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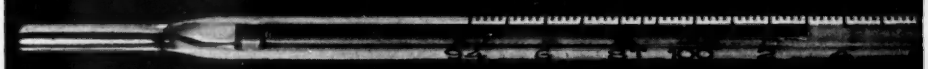

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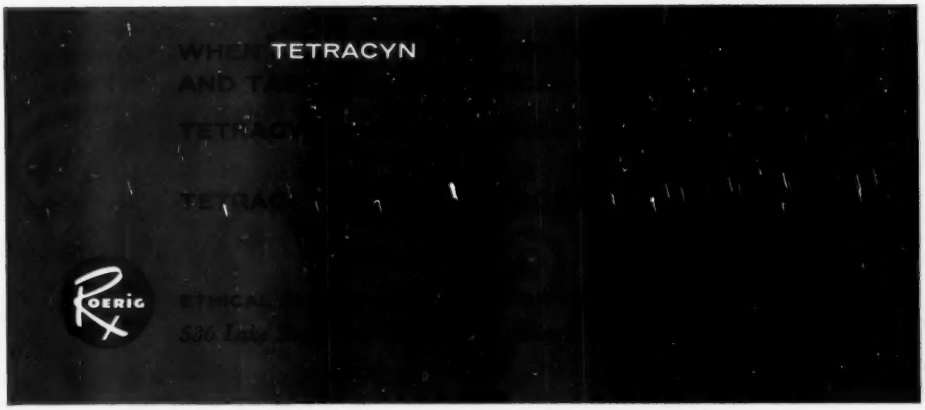

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1. Martin, G. A., Garfinkel, B. T., Brooke, M. M., Weinstein, P. P., and Frye, W. W.: *J.A.M.A.*, 151:1055, Mar. 28, 1953.
2. Kohn, J.: *Jour. Trop. Med.*, 53:212, Nov., 1950.
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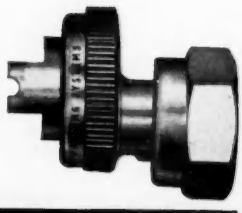

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1. Lillie, R. D., Histopathologic Technic 1948, pp. 157-160.
2. Chiffelle and Putt, Stain Technology, 26:51, 1951.

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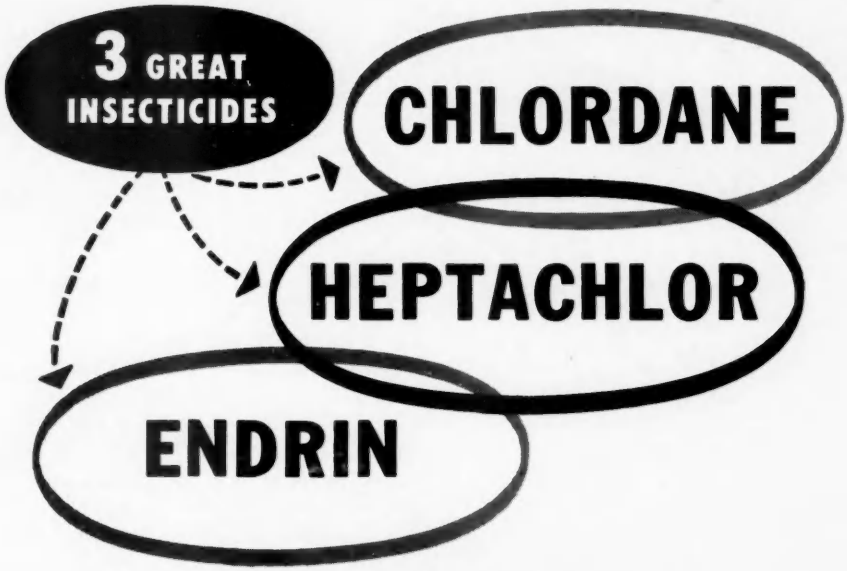

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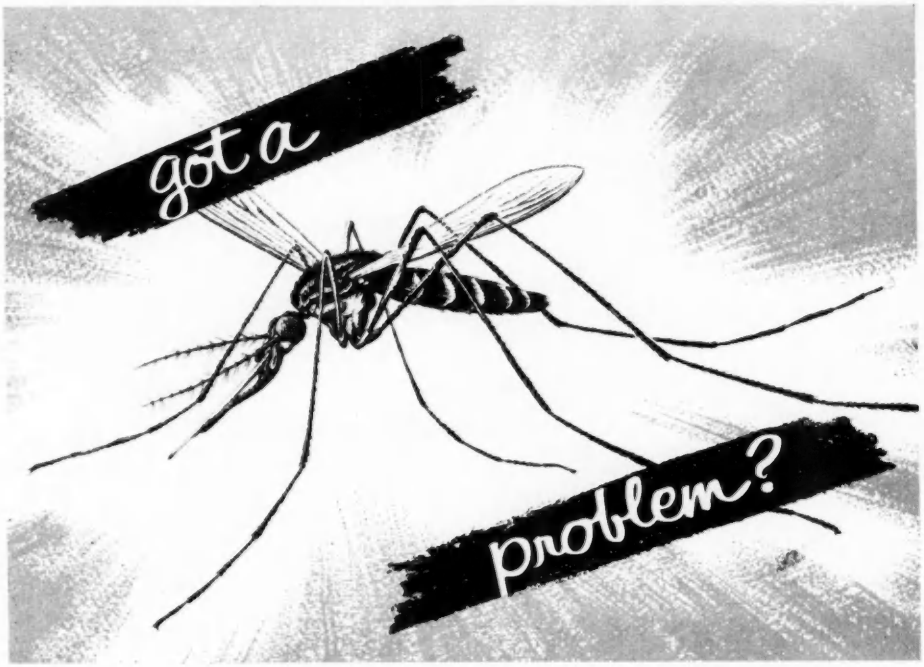

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AN OUTBREAK OF MALARIA IN CALIFORNIA, 1952–1953

ROSEMARY BRUNETTI, ROY F. FRITZ AND ARTHUR C. HOLLISTER, JR.

Bureau of Acute Communicable Diseases, California State Department of Public Health, Berkeley, California, and Communicable Disease Center, Public Health Service, Atlanta, Georgia

The recent occurrence of an outbreak of malaria in California dramatically challenged the concepts of malaria prevention and control in the United States. The rapid decline of the incidence of this disease in the United States has fostered the hope that malaria can be eradicated from this country (Young, 1953). The appearance of this outbreak does not derogate from this goal but it does point out that, given the right set of circumstances, malaria still can be transmitted here.

This was the first outbreak of malaria to be recorded in the United States since 1945 and it offered a rare opportunity to observe the natural history of *Plasmodium vivax* in a nonendemic area and in a completely susceptible population.

METHODS OF STUDY

Attention was first focused on the outbreak in August, 1952. Three illnesses in young girls were diagnosed as *P. vivax* malaria and reported to the California State Department of Public Health from three separate local health jurisdictions in Central California. These three simultaneous reports of malaria spurred the State Health Department to action. A preliminary investigation confirmed these three cases as malaria and indicated that all were Camp Fire girls who had recently vacationed at Lake Vera, Nevada County. The probability that a focus of infection existed there and the possibility that additional cases would appear precipitated an immediate and extensive investigation.

In reality the investigation of this episode was biphasic. The first part was conducted in the fall of 1952 when the outbreak became apparent. The second phase took place in the spring and summer of 1953 when additional cases having the same source were observed. The fall activities localized the place of infection, identified the source and defined the population at risk. The 1953 activities were concentrated on finding additional cases, the clinical aspects of the illness, observations on periods of latent infection and on efforts to prevent a reoccurrence during the 1953 camp season. The methods used in the investigation in 1952 were simply extended into 1953.

The operational plan and its execution was a cooperative enterprise coordinated by the State Health Department and the Public Health Service. The Camp Fire officials furnished data on the camp organization and activities, and also supplied a list of approximately 1,500 persons known by them to have been at Lake Vera during the summer of 1952. Visits to the permanent residences and summer homes in the area added another 100 persons to the total

population presumed to be at risk. These data not only provided the necessary identification of each camper but also gave the dates spent at camp and a medical record for this period. They greatly facilitated the observation of this exposed population for latent cases during the summer of 1953. The details of the camp activities, coupled with current and previous environmental reports of the lake, permitted an appraisal of the circumstances contributing to the transmission of these infections.

The local health officers, with the cooperation of the local medical societies, alerted private physicians, assisted in the investigation of each suspected case, searched for possible sources of infections within their jurisdictions, released publicity to local newspapers, and circularized the parents and guardians of campers regarding the situation. State and Federal personnel were actively engaged in epidemiological investigations and in entomological and topographical surveys of the area. Assimilating pertinent records was also a part of their activities. This work included a perusal of the reported cases of malaria in the State Health Department for a clue to the possible source, a comparison of previous entomological reports with current findings, and a summation of the malaria records of Nevada County as well as those of the seven counties where the cases resided. The assistance of local mosquito abatement districts and of persons expert in malariology was also readily forthcoming.

Clinical and epidemiologic investigation of each suspected case verified the diagnosis, eliminated the possibility of other exposures, documented the incubation period, and provided for a clinical evaluation.

FINDINGS

Camp location and organization. The Camp Fire Councils of four central California cities, Sacramento, Vallejo, Oakland and Piedmont, maintain summer camps at Lake Vera (Fig. 1). These councils draw their membership from six of the counties where most of the cases resided. Lake Vera is a 15-acre artificial impoundment located in the rolling foothill terrain of the Sierra Nevada mountains at an elevation of 2,400 feet. Aside from the four camps mentioned, there are two privately owned summer homes on the lake front. The area around the lake is sparsely settled and only two of the nearby 18 permanent residences are within a half-mile radius of the camps.

The camps opened early in June and closed early in August. The usual session lasted from one to two weeks but some campers and most of the staff remained in attendance for the whole season. The census for each session varied for each camp, with an over-all average of approximately 350 persons.

A few of the staff members had permanent sleeping quarters; all other campers slept in the open on cots or in sleeping bags. This outdoor living provided ample exposure to the innumerable mosquitoes present at the lake.

The Bureau of Vector Control of the California State Department of Public Health surveyed the area early in September, 1952. Their findings confirmed a similar survey conducted by them in June, 1950. Mosquitoes were abundant. The terrain of this lake provided many excellent breeding places for *Anopheles*

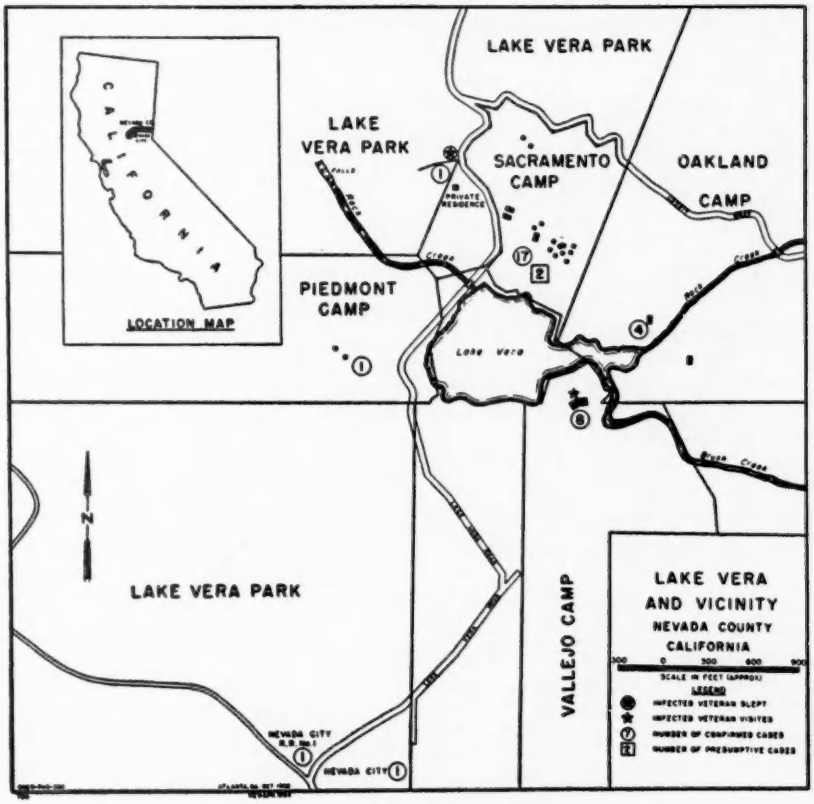

FIG. 1. Area where infection occurred

punctipennis, *Anopheles freeborni*, *Culiseta incidens* and *Culex tarsalis*. The most prevalent mosquito found in September, 1952, was *A. punctipennis* with slightly fewer *C. incidens* and *A. freeborni*. Some anopheline mosquitoes were captured for dissection but none were found to contain the malaria parasites (Records, 1952; Records, 1950).

Occurrence of cases. From August, 1952, to August, 1953, a total of thirty-five cases of *P. vivax* malaria were recognized in the total group of 1600 persons. Sixteen lived in Sacramento County, seven in Solano County, four in Contra Costa County, three in Alameda County, two in Nevada County, one each in Amador and Yolo Counties and one in the city of Reno, Nevada (Fig. 2).

All of these were primary attacks and the only possible common place of contraction and common time of exposure was their presence at Lake Vera during the summer of 1952. All but two of the cases were confirmed by the finding of *P. vivax* in blood smears. Blood smears were taken on these two cases,

FIG. 2 Cases of malaria by place of residence

but no plasmodia were noted and the slides were destroyed. However, since both these cases were epidemiologically related to the others and their clinical illnesses were compatible with the diagnosis, they are therefore included in the report.

Age and sex distribution. The age and sex distribution reflected the population at risk. Twenty-seven of the cases were vacationing girl campers, ages 9 to 19. Four of the adult females were members of the staff. The other adult female was a summer resident at Lake Vera. One of the males was a volunteer worker

TABLE 1

Cases of malaria, by age and sex

Age Group	No. of Cases				
	Girl campers and staff		Local residents		Total
	Male	Female	Male	Female	
5–9	0	3	0	0	3
10–14	0	16	0	0	16
15–19	0	8	0	0	8
20 and over	1	4	2	1	8
Total	1	31	2	1	35

at the Vallejo camp and the other two were permanent residents of the area (Table 1).

Clinical findings. The classical malaria symptoms of chills, fever and sweating were experienced by all but one of the cases sometime during the course of illness. The one exception was a 60-year old female who only had daily bouts of fever for 10 days. This case was confirmed by laboratory two days after onset and her fever persisted for eight days in spite of immediate therapy with chloroquine.

Eighteen of the patients noted premonitory symptoms one to seven days before their first rigor. These were chiefly lassitude, headache, arthralgia, nausea, vomiting, chilliness, and fever. A prodromal stage of intermittent bouts of pharyngitis, fever, headache, and chilliness occurred in two other cases 41 to 48 days before the first typical paroxysm. Although temperatures to 106°F. were not uncommon, the outstanding complaint was the arthralgia of the lower extremities. In the majority of the patients the paroxysms consistently occurred in the late afternoon.

Source of infection. The dates of onset of the eight original cases presented valuable clues to the dates of exposure (Fig. 3). The grouping of the onsets early in August suggested an exposure to infective mosquitoes in mid-July. The camp records revealed that all of the infected girls had been at Lake Vera sometime between July 15 and July 27. Although some had been there prior to this time, and some after this time, this period was the only common date at camp. Assuming the extrinsic incubation period to be 12–14 days (71°–75°F.) and the intrinsic incubation period to be 11–14 days (Boyd, 1949), it was speculated that mosquitoes most likely were infected at least 23 days prior to first onset of illness. Considering the cold weather during June, the most probable period for mosquito infection was estimated as the first 10 days of July.

An intensive search was conducted to locate the source of this outbreak. Since several hundred cases of malaria were being reported in the State among servicemen who had contracted the disease in Korea, an especial effort was made to locate all such persons who had been in the Lake Vera area. Through a fortuitous circumstance such a serviceman, a recently discharged marine, was

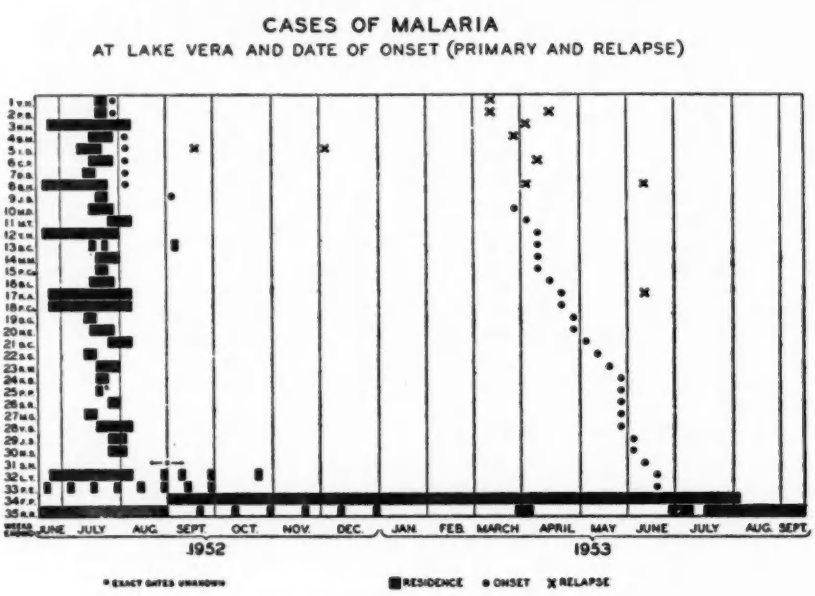
CASES OF MALARIA
AT LAKE VERA AND DATE OF ONSET (PRIMARY AND RELAPSE)
JUNE JULY AUG. SEPT. OCT. NOV. DEC. JAN. FEB. MARCH APRIL MAY JUNE JULY AUG. SEPT.
1952
1953
RESIDENCE
ONSET
RELAPSE

FIG. 3

located. He had returned from Korea in November, 1951, and had experienced his first attack of malaria in April, 1952. He had spent the 1952 Fourth-of-July weekend at Lake Vera and while there had suffered a relapse of his malarial infection. His hospital records confirmed that both of these attacks were caused by *P. vivax*.

Although no other possible source of the Camp Fire girls' infections could be found among persons known to have visited the lake, including several other servicemen, or among permanent residents, the possibility of there being other sources cannot be denied. The camps are located in a vacation area where many persons obtain recreation each year. However, the presence of this infected ex-serviceman at the lake early in July, his acute relapse, and the onsets of illness due to malaria in the patients coincide with the accepted timing for infection of and transmission by the vector (Russell, 1952; Boyd, 1949).

INCUBATION PERIODS AND LATENCY

Recognizing that this Korean strain of *P. vivax* may exhibit a latent period of from 6 to 10 months (Alving *et al.*, 1952), the appearance of a few additional cases in the spring of 1953 was anticipated. This speculation was tempered by the possibility that suppressive therapy may have contributed to the latency exhibited by the Korean veterans (Young, 1952; Edit. 1951) and by the improbability of many more persons becoming infected following such a limited exposure of mosquitoes to only one source. Therefore the occurrence of 26 latent cases in 1953 was beyond expectation.

The first latent case appeared late in March, ten more cases occurred in April, followed by eight in May, five in June, and one each in July and August (Fig. 3). This distribution of dates of onset supported Korteweg's hypothesis that significant numbers of *P. vivax* infections contracted in autumn may remain clinically latent until the next year (Swellengrebel and De Buck, 1938).

The most significant contribution of this outbreak is perhaps the documentation of the incubation periods. The Camp Fire records provided the exact dates of attendance at camp on thirty-one of the cases. Similar but less reliable information was obtained by interview from the four nonmembers. The dates of onset were confirmed by medical evaluation of each case (Fig. 3). By matching these dates alone, incubation periods from 10 to 365 days could be estimated. However, the knowledge that the veteran spent only July 4, 5, 6 and 7 at the lake made it possible to estimate the time infective mosquitoes were active and thereby more accurately calculate the human incubation periods.

The survival and development of the *Plasmodium* in the mosquitoes is dependent on the environmental temperature. The generally accepted 12-to-14-day cycle requires temperatures between 71° and 75°F. (King, 1929). Grassi (Boyd, 1949) noted that *P. vivax* failed to develop in the mosquito if the environmental temperature was below 63.5°F. for the first few hours after biting. If the temperature is between 82° and 86°F. maturation of the sporozoites may be shortened to 8 days. Conversely, at temperatures between 59° and 62°F. this cycle of the parasite may be lengthened to 38 days (Boyd *et al.*, 1935). The average temperature in Lake Vera area in June, 1952, was 58°F. The average for July was 70.2°F. (U. S. Department of Commerce, 1952). The establishment of the parasite in the mosquitoes in June would be improbable but would be possible in July. Therefore, infective mosquitoes were most likely active by July 16th, 12 days after the veteran's visit.

Two additional observations establish the significance of this date. No cases of malaria appeared among the 524 campers who attended only the sessions conducted before July 15th. All of the cases occurred in campers who were at the lake after this time (Fig. 3). Twenty-nine of the cases did not return to Lake Vera after the camps closed early in August, and these cases provide the most reliable data on incubation periods. All of them had been in camp sometime between July 15 and 28. This information, coupled with the proved dates of onset, permitted an accurate estimate of their incubation periods.

The shortest incubation period was estimated as 10 days and the longest as 316 days. The nine 1952 cases became ill in from 10 to 40 days after exposure (eight in less than 14 days, and one in 40 days). The latent cases exhibited incubation periods ranging from 217 to 316 days. Six of the original nine cases experienced relapses at the same time that new cases were appearing, that is, at 226 to 255 days after being bitten by the infecting mosquitoes.

Speculation on the dates of exposure of the six non-Camp Fire members indicated that they also became ill 7 to 10 months after infection. One of the males (F. P.) moved to the lake area early in September 1952; he became ill 10 months later, in July 1953. However, he visited the area during the summer preparatory to moving. The other resident of the area (S. H.) made frequent

visits to the lake after the camps closed in August. He became ill in June, also 10 months later. Two others (B. C. and P. E.) were volunteer workers at the Vallejo Camp who spent periodic weekends at the lake. They became ill 7 months after their last visit. The other two (L. Y. and R. P.) could have been infected at any time from mid-July until mid-Septemger. The shortest incubation period for them would be 9 months and the longest 12 months.

Control measures. Prevention of a similar occurrence during the 1953 season was of paramount importance. As soon as the nature of the outbreak was recognized, the area was sprayed and plans for permanent control measures were formulated.

Before the opening of the 1953 season, most of the major breeding areas of Lake Vera were eliminated. During the summer season surveillance of the area was continuous and DDT sprays were used to keep mosquitoes at a minimum. The results of these activities were excellent. No *Anopheles* were found while the camps were in session and *Culiseta* and *Culex* species were practically absent.

DISCUSSION

Some temperate zone strains of *P. vivax* are known to exhibit incubation periods from 6 to 10 months (Coatney *et al.*, 1950). Since the Korean strain belongs to this group, these twenty-six long-latent cases were not unusual in this respect. Nor was this single-source outbreak unprecedented. A single-source outbreak occurred in Contra Costa County, California during 1945 (Records, 1945). A similar episode took place (van Soest and van Thiel, 1953) in a home for the aged in the Netherlands. Coincidentally, there also, 35 cases of *P. vivax* were related to a single infective case. Twenty-nine of these exhibited incubation periods of "several months" duration. Six of the cases were considered to be recent infections.

The uniqueness of this California outbreak is that it occurred in a nonendemic area in a nonimmune population which had not been subjected to suppressive therapy. The Netherlands' outbreak occurred in an endemic area which tends to make it difficult to eliminate completely other possible sources of exposure.

Suppression of the primary attack through the use of prophylactic drugs has been considered to be a factor in the latency demonstrated by the Korean veterans (Young, 1952; Edit. 1951). Since none of the California cases were given suppressive therapy, their long incubation periods cannot be attributed to this factor. These delayed primary attacks could have been the result of a low sporozoite dosage (Coatney and Cooper, 1948). Unfortunately, this could not be confirmed. Many of the patients who experienced their first attack months after exposure complained bitterly about mosquito bites. On the other hand, many of the cases having short incubation periods could not recall being bitten. The length of time spent at camp could not be correlated with the early or delayed primary attack.

Swellengrebel and De Buck (1938) in their studies in the Netherlands felt that autumnal anopheline infection was responsible for most of the malaria cases appearing throughout the year. This outbreak supports that observation.

Directing current investigational activities to possible exposures 7 to 10 months prior to onset is as important in locating the source of spring and early summer attacks as is inquiry concerning the patient's recent whereabouts.

SUMMARY

Thirty-five cases of *P. vivax* malaria occurred among a group of Camp Fire girls who attended the 1952 summer camp at Lake Vera, Nevada County, California. Nine of the cases experienced primary attacks 10 to 40 days after exposure. Twenty-six exhibited latent periods of 217 to 316 days. The probable source of these infections was traced to a recently returned Korean veteran.

The occurrence of this outbreak demonstrated the vulnerability of this country to the introduction of malaria from abroad when favorable circumstances exist, and it provided an unusual opportunity to observe the natural history of the *P. vivax* strain of malaria in a nonimmune population living in a nonendemic area.

The investigation confirmed, extended, and elucidated the knowledge on latency and delayed primary attacks exhibited by certain strains of temperate zone malaria. The documentation of the incubation periods substantiated the observation that many *P. vivax* infections contracted in autumn may remain clinically latent for eight to nine months.

ACKNOWLEDGMENTS

The authors are appreciative of the cooperation of the Camp Fire executives and of the assistance of many persons in the epidemiologic investigation. Nurse-epidemiologist Naida Sherwin and Albina Bozym of the California Department of Public Health and the U. S. Public Health Service, respectively, carried out many of the personal interviews required. Drs. Henry G. Mello, Health Officer of Vallejo and Solano County; Frank L. Kelly, Health Officer of the City of Berkeley; J. C. Geiger, Health Officer of the City of Oakland; James C. Malcolm, Health Officer of Alameda County; Henrik L. Blum, Health Officer of Contra Costa County; David Frost, Health Officer of the City of Alameda; and Ira Church, Health Officer of Sacramento County, in whose jurisdiction the majority of persons at risk resided, assisted materially in the investigation.

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MALARIA CONTROL AT LAKE VERA, CALIFORNIA, IN 1952–53

RUSSELL E. FONTAINE, HAROLD F. GRAY AND THEODORE AARONS

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The outbreak of malaria at Lake Vera in Nevada County, California, in 1952 (Brunetti *et al.*, 1953; Brunetti, 1953; Gray, 1953, 1953a; Fontaine, 1953) made it imperative that control measures be taken to prevent a recurrence of the disease in the following year. Not only was it desirable that the health of nearly 1,800 persons be protected, but the Oakland, Piedmont, Vallejo and Sacramento Councils of the Camp Fire Girls had a considerable investment in equipment and facilities at the four camps which would be jeopardized by a recurrence of the outbreak. In the absence of an effective local health department in this county and because of the wide area of the State from which the occupants of the four camps came, the State Department of Public Health initiated the epidemiological studies and the necessary control work. The Alameda County Mosquito Abatement District participated for the reason that about one-half the population of the camps came from that county, and also because of the experience of its Engineer-Manager with the malaria problem in Northern California, extending back to 1910.

Malaria had been endemic in Nevada County since the "gold rush" days of the 1850's, but after about 1930 had been absent from the area. Even the return of presumably infected military personnel from World War II did not result in any indigenous cases of malaria being reported. The malaria died out without any general attempts having been made at mosquito control in the county, although a small project at North Columbia was carried out by the Nevada Irrigation District under the direction of Gray in July, 1918. An intense endemic malaria in this area was stopped by drainage to eliminate an *Anopheles*-producing seepage area below an irrigation main canal.

MOSQUITOES AT LAKE VERA

Mosquitoes have been quite numerous in the Lake Vera area. One of Gray's daughters attended this camp in 1931 and complained about the mosquitoes. In a letter dated October 19, 1949, the Executive Director of the Oakland Council, in asking for a survey by the State Department of Public Health, said that the area had been sprayed (materials not stated) that year, and possibly in previous years, by airplane and by hand, with little success. In June, 1950, acting on this request, Harvey I. Magy, Vector Control Specialist of the California State Department of Public Health, made a report to his Chief on the mosquitoes at Lake Vera. The following adult mosquitoes were captured and identified, in the order of relative prevalence: *Aedes varipalpus*, *Aedes increpitus*, *Culiseta incidens*, *Culex tarsalis*, and *Anopheles punctipennis*. Larvae

recovered were *Culex tarsalis*, *Culiseta incidens*, and *Anopheles*, either *punctipennis* or *freeborni*, which are taxonomically not distinguishable in the larval stage. Mr. Magy made recommendations for various control measures, but apparently no effective action was taken by the Camp Fire Girls' organization.

In July, 1952, as was subsequently discovered by the epidemiologists (Brunetti and Fritz, 1954), a returned veteran from Korea while camping near the lake, suffered an acute relapse of *vivax* malaria, and infected a considerable number of Anopheles mosquitoes, resulting in 35 cases of malaria among the Campfire Girls.

On August 30, 1952, following the first reports of malaria cases by the Epidemiology Section, Bureau of Acute Communicable Diseases, California State Department of Public Health, Fontaine made an entomological survey of the area, which was supplemented on subsequent visits. He found relatively high densities of *Anopheles* larvae (averaging 12 per dip) in the shallow margins of the lake, especially where there was emergent vegetation or where willows or brush extended from the banks over or into the lake margin, or in floating algae mats. Such larval habitats extended almost completely around the lake and covered about one-quarter of the lake's surface. Adult mosquitoes of the following species were collected: *Anopheles punctipennis*, *Anopheles freeborni*, *Aedes varipalpus*, *Culiseta incidens* and *Culex tarsalis*. All of the washrooms, toilet and shower buildings were favored resting stations for anophelines, with *A. punctipennis* being the dominant species.

A reconnaisance for several miles radially from the lake was made without disclosing additional larval sources of significance. A few anopheline larvae were found in stream bed pools in Rock Creek and Brush Creek, tributaries to the lake, and in a few small marshy areas within a mile of the lake moderate numbers of anopheline and *Culex* larvae were found.

Early in 1953, before the lake was filled, mosquito larvae (Culicines) were present in pools in the lake bottom.

DESCRIPTION OF THE AREA

Lake Vera is a water impoundment at the junction of Rock Creek and Brush Creek on the westerly slope of the Sierra Nevada mountains in Nevada County, California, about 5 miles northerly from Nevada City, at an elevation of 2,500 feet. Impoundment is by a low concrete dam with removable wood flash boards. The maximum depth is about 10 feet and the superficial area is approximately 15 acres. The flash boards are placed during May to fill the lake, and removed in late September to drain it.

Emergent vegetation in the shallow marginal areas consisted of spike rush (*Juncus* sp.) and tules (*Typha* sp.). Willows and deer bush lined the shore areas, except where cleared for the use of the campers. Floating mats of algae appear in July and increase in magnitude through the summer.

The general area is one of grassy foothills with moderate to steep slopes with narrow valleys or gulches. The earth consists of sedimentary deposits with some rock exposures. Ground cover consists of a transitional forest, mainly pines,

with some oaks, maples and other deciduous trees, and brush. Fairly heavy rains occur from October to May, with occasional snow. From May to October rainfall is practically absent, and the daily temperatures are high (up to about 100° F.) with moderately warm nights. Stream flow diminishes rapidly during the summer, leaving marginal and stream bed pools in many places. Gold mining, lumbering, fruit growing and stock raising are the principal activities in the county. Fruit growing is under irrigation.

CONTROL OPERATIONS

Since the camp was closed for the year at the time of the survey by Fontaine on August 30, 1952, the recommendations for mosquito control were based on the idea of prevention of a recurrence of the epidemic in 1953.

Preliminary plans for these operations were made at a conference on September 22, 1952, between the executives of the Camp Fire Girls' Councils, and R. Fritz of the U. S. Public Health Service; R. F. Peters, Chief, Bureau of Vector Control, California State Department of Public Health; and Gray. With nine cases of malaria recognized during 1952, the executives of the Camp Fire Girls' Councils were quite concerned over the prospect, and when an additional 26 delayed cases occurred early in 1953, they were sufficiently frightened to be willing to follow effectively the recommendations made as to control operations. Basically, our recommendations consisted of three parts: (1) clearing the marginal areas of the lake, and either filling shallow areas, or deepening them to at least a four foot depth, according to the topography and minimum cost; (2) application of DDT residual sprays to all buildings and other possible shelters, to kill off the over-wintering *Anopheles* mosquitoes; (3) larvicidal operations in the spring and summer of 1953, within a one mile radius of the lake, including a delay in filling the lake until the latest practicable date. In addition, for the general comfort of the campers, we recommended that a project of tree-hole filling with sand and cement be undertaken to eliminate the very obvious pest of *Aedes varipalpus*. Water level fluctuation by changing flash boards in the dam was considered, but ruled out as impracticable and an unnecessary interference with the use of beach and swimming facilities. It was also suggested that the lake be stocked each summer with *Gambusia affinis*.

The shore line improvement was done with a hired bulldozer in May of 1953 before the lake was filled. It was generally well done, but a few small shallow areas in Brush Creek were missed. These were to be filled in the spring of 1954, and additional work was to be done to "smooth up" the 1953 fills or cuts.

The residual spray project was carried out during September–October, 1952, using 25 gallons of 25% DDT emulsible concentrate applied as a 5% emulsion. In the spring of 1953 mosquito adults were practically absent in the treated structures, as compared with the numerous adults found there on August 30, 1952. Inspections on February 10 and March 2, 1953, disclosed no *Anopheles* adults, and on April 20 four *Anopheles* adults were seen, of which one was captured and identified as *A. freeborni*. Initial larviciding was begun on May 3, 1953, by the camp counsellors, but on May 19 Fontaine recovered larvae in

pools in the lake bottom, averaging 5 *Anopheles* per dip. On June 19, three days after the lake was filled, Culicine larvae were taken in localized shore line areas.

The 1953 larviciding campaign was begun on June 19–20 when Gray took M. Freeman, a high-school student who had been trained for a few days in the Alameda County Mosquito Abatement District, to Lake Vera. The District loaned some spraying and collection equipment and provided DDT emulsible concentrate. Inspections on June 20 disclosed no *Anopheles* larvae, but found about a ¼ acre shallow patch in Brush Creek which was swarming with *Culiseta* larvae. No *Anopheles* adults were observed, but *Aedes varipalpus* adults were moderately abundant.

The spray used was a 2½% DDT emulsion, applied by hand sprayer equipment. The inspection and spraying operations were supervised by occasional visits by Fontaine and Aarons during the summer, and were effectively carried out, as *Anopheles* adults were practically absent during the entire camping season, and *Culex* and *Culiseta* were neglibible. A few *A. punctipennis* adults were captured on July 1, and one *A. freeborni* adult on July 14. On July 22 the State Department of Public Health applied an aerosol treatment to the area to reduce the *Aedes varipalpus* population. Larviciding operations ceased on August 14, when the camps were closed. Three days later a few *Culex tarsalis* and *C. stigmatosoma* adults were found in tents and cabins. Anopheline adults were still absent on August 28, but numerous *Anopheles* larvae were found in pools along the stream margins, in addition to *C. tarsalis*, *C. boharti* and *Culiseta inornata*. The last observation of the season was made on September 11, 1953. A very sparse *Anopheles* density was observed along the lake margin; *C. tarsalis* and *C. boharti* larvae were found in fairly large numbers, but no *Anopheles* adults could be found.

The operation appears to have been a complete success, in that no new cases of malaria had appeared among the Camp Fire Girls during the camping season nor up to the end of January, 1954.

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THE FIRST LARGE AREA IN THE TROPICAL ZONE TO REPORT MALARIA ERADICATION: NORTH-CENTRAL VENEZUELA

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This paper reports the eradication of malaria in a large tropical area of Venezuela, the first country to organize a nation-wide campaign against malaria with DDT (Russell, 1952, 1952a). Our use of the term "malaria eradication" is based upon the principle advocated in the "Final report of the U.S.A. National Malaria Society Committee on criteria to determine when malaria ceases to be an endemic disease" (Committee report, 1951). Some data on the epidemiology of malaria in Venezuela and on the organization of the service have been presented previously (Gabaldon, 1949, 1951; Berti, 1949).

Before DDT was introduced in 1945, malaria control activities in Venezuela were limited to (a) universal free distribution of quinine and quinacrine; (b) large drainage and filling operations around towns; and (c) use of larvicides (Paris green) and imagocides (mostly pyrethrum), particularly in epidemic areas. The drug distribution service, established in 1937, was probably the first of its type to make antimalarials available without payment to rich and poor alike. In one year, more than 800,000 individual treatments were given. Drainage works consisted mostly of paving canals with precast concrete slabs, some towns requiring more than 50 kilometers of ditches. *Anopheles albimanus* and *A. darlingi* were successfully controlled by this method, the disease due to the last species being eliminated along with the mosquito for the first time from several towns (Berti, 1945). Imagocides were used on a rather large scale, more than 60,000 liters of the mixture being sprayed in one year.

The progress in DDT spraying since 1949 is indicated in Table 1. Other insecticides were used during the last three years. BHC was employed as wettable powder generally mixed with wettable powders of DDT, to spray houses in zones heavily infested with triatomids. Chlordane was used in solutions or emulsions to assist in the control of house flies. Dieldrin was employed in emulsions to control triatomids mostly in the zone where malaria has been eradicated. In 1952 the number of houses sprayed with this insecticide was 7911, probably the largest program of indoor residual spraying with dieldrin carried out so far.

The area in which malaria has been eradicated, or is in process of eradication (Area I) includes about 180,000 square kilometers, representing 30 per cent of the old malaria zone, and 20 per cent of the Venezuelan territory. This area occupies the northern and central portions of Venezuela, where DDT spraying was first organized. The total number of inhabitants of the area is 2,430,986, or 49 per cent of the population of Venezuela.

Venezuela is usually divided into three regions, Costa-Cordillera, Llanos and Guayana (Fig. 1, A, B, and C). These regions are not only topographically, eco-

TABLE 1

Progress of DDT spraying program in Venezuela

	1949	1950	1951	1952	1953
Localities sprayed	5,771	10,511	16,224	21,474	23,400
Average number of houses in each	62	43	31	25	23
Sprayings per year	1.6	1.5	1.6	1.6	1.6
Number of houses protected	356,981	450,581	495,973	529,874	548,304
Number of house-sprayings	585,434	675,250	800,661	851,226	877,287
Number of persons protected	1,697,903	1,948,744	2,134,939	2,172,500	2,248,046
DDT used (kgms. technical)	277,601	314,336	407,884	408,874	382,766
BHC used (kgms. gamma isomer)	114	1,832	2,599	8,161	20,138
Chlordane (kgms. technical)	—	18,851	41,586	4,935	1,465
Dieldrin (kgms. technical)	—	19	—	1,559	12,650
Cost per inhabitant (Bolivars*)	1.70	1.71	1.64	1.76	1.64
Houses to be sprayed	507,773	536,758	599,758	630,951	630,951
Percentage of houses sprayed	70.3	84.0	82.7	84.0	87.0

* 1 bolivar = US $ 0.30.

FIG. 1. Map of Venezuela to show: (a) the political divisions of the country; (b) the three geographical zones in which it has been divided, A. Costa-Cordillera, B. Llanos, and C. Guayana; and (c) the different areas of present malaria prevalence of which Area I is the area of malaria eradication.

nomically and demographically different, but they also vary malariologically. Area I, the area of malaria eradication, is in the central portion of the Costa-Cordillera and Llanos regions. Outside Area I, we have divided Venezuela malariologically into four other areas as shown in Figure 1: Area II, with less than 0.1 per cent of the malarious zone of the country, where 28.0 per cent of the malaria cases observed in 1952 were found and where *A. aquasalis* is the vector; Area III, with 56.6 per cent of the malarious zone of the country and 14.7 per cent of the 1952 malaria cases registered in Venezuela, and where *A. albimanus*, *A. albitarsis* and *A. darlingi* are the vectors; Area IV, with 3.3 per cent of the malarious zone of the country, with 50.6 per cent of the malaria cases identified in the country in 1952, and where *A. darlingi*, *A. nuñez-tovari*, and *A. pseudopunctipennis* are the vectors; and Area V, with 5 per cent of the malarious zone of the country, where 5.6 per cent of the malaria cases of the country were observed in 1952, and where *A. albimanus* and *A. darlingi* are the vectors.

EPIDEMIOLOGY OF MALARIA

In Area I malaria was highly endemic in some places and highly epidemic in others, so that the disappearance of the disease following DDT spraying was not influenced at all by any low prevalence of the infection. A general review of the epidemiology of malaria in this area seems advisable in order to give a clear idea of what has been achieved.

Vectors. The two main vectors of Venezuela, *A. albimanus* and *A. darlingi*, were responsible for the most of the transmission in Area I. Four species considered the secondary vectors of the country, *A. albitarsis*, *A. aquasalis*, *A. nuñez-tovari* and *A. pseudopunctipennis*, are also present, but with the exception of the first, did not play any apparent rôle in transmission.

Malaria parasites. Plasmodium falciparum was the predominant species of parasite in Venezuela but *P. vivax* was more common below five years of age. The age prevalence of those with parasites indicates that the highest incidence is found in ages 2 to 4, but *P. vivax* decreases with age more quickly than the other two parasites. The index numbers of the seasonal distribution of the parasites, taken Janauary as 100, are generally above this figure during the second six months of the year (Gabaldon, 1949). These facts are presented in order to evaluate the significance of the present parasite prevalence in Area I.

Constitution of malaria. The term "constitution of malaria" means the tendency of this disease to change prevalence from year to year in a given area, as measured by annual variations in the spleen index independent of seasonal changes. This term defines trends of endemicity or epidemicity in a zone measured by (a) the *ratio of endemicity*, or the lowest spleen index observed in a 5-year period divided by 5, the assumed normal figure of this index; and (b) the *ratio of epidemicity*, or the highest spleen index observed in such period divided by the lowest (Gabaldon 1946).

In Venezuela holoendemic malaria is unknown, but hyperendemic malaria was present in several municipios, although it was not the most common type of the disease. In Area I several municipios (secondary political subdividisions of

States) had ratios of endemicity above 10, and some even above 15. In these municipios the general death rate was generally between 30 and 50 per 1,000, but most endemic districts had ratios of endemicity below 8, with general death rates from 20 to 25. The highest endemicity was mainly due to *A. darlingi*, but some high ratios were found in *A. albitarsis* territory. *A. albimanus* was generally responsible for moderate endemicity.

In Area I, as in most of Venezuela, malaria showed a definite trend to epidemicity. Regional epidemics due to *A. darlingi* or *A. albimanus* alone or by both species were common during the ascending limb of the para-quinquennial cycle characteristic of the disease in Venezuela (Gabaldon, 1948, 1949). *A. albimanus* was also responsible for some localized epidemics connected with rice cultivation observed in Area I (Berti and Montesinos, 1945) and for epidemics connected with excessive rainfall. Regional epidemics in this area were very serious, total death rates between 60 and 70 per 1,000 being observed in several municipios during epidemic years. As late as 1941, malaria death rates were as high as 531 and 1,125 per 100,000 inhabitants in the most afflicted States, Carabobo and Cojedes. There was no other disease, even influenza in 1918, which caused a higher mortality than epidemic malaria in Venezuela in the period 1905–1945 (Gabaldon and de Pérez, 1946).

What has been said above clearly indicates that malaria has been a very important disease in Venezuela—its greatest public health problem. This is emphasized in order to evaluate adequately the meaning of the elimination of the disease in Area I.

ERADICATION OF MALARIA

Area I, the area of malaria eradication in North-central Venezuela, is made up of two sections (a) one of about 20,000 sq. kms. called Area Ia, where no primary indigenous cases of malaria, with the possible exceptions of four discussed below, have been found since 1949, and (b) another of about 160,000 sq. kms., called Area Ib, where only two primary indigenous cases of malaria were found after 1951. These areas are relatively small (although larger than England and Wales) but in terms of inhabitants, they represent almost one-half of the total population of Venezuela. In Area Ia malaria has been eradicated according to the very strict application of the definition given above. In Area Ib all available evidence indicates that malaria is on the road to eradication. A review of the data on which these conclusions are based is presented here.

Search for malaria cases. Three methods have been used to discover malaria cases, all of which are based on microscopic diagnosis. These are (a) cases reported by doctors, (b) cases found by health visitors in house visits to malarious districts, and (c) cases found in the examination of children for spleen indices made every year in selected places. The results obtained by these methods are presented in Table 2.

In Area I there are 1782 doctors of whom 590 are in towns of the malarious municipios, which means that these municipios have one doctor per 2608 inhabitants. Medical care in the municipios which never had malaria is much better, as

TABLE 2

Results of the three methods used in search of microscopically diagnosed malaria cases in Area I

Data	1950	1951	1952	1953
Clinically diagnosed malaria cases reported by doctors	6,819	2,649	830	182
Slides taken to confirm diagnosis or from other patients with fever	3,621	2,383	5,011	3,469
Number of these slides positive for plasmodia	70	17	16	12
Number of house visits made by health visitors	136,242	174,455	163,253	147,185
Suspected cases from whom slides were taken	2,562	2,789	2,941	4,729
Number of these slides positive for plasmodia	77	20	10	8
Positive slides per 100,000 house visits	58	11	6	5
Number of children examined for spleen indices	8,196	7,913	5,721	6,300
Number of children with splenomegaly	589	292	182	155
Number of blood smears from children with or without splenomegaly	3,165	2,953	1,520	2,365
Number positive for plasmodia	0	1	0	0
Total number of slides examined in Area I	9,348	8,125	9,472	10,561
Total number of microscopically diagnosed cases in Area I	147	32	22	20

there is one doctor per 750 inhabitants. These physicians report weekly to the Division of Epidemiology and Vital Statistics of the Ministry of Health and Social Welfare, the number of cases which they diagnose as malaria. Most of these are based on clinical diagnosis only. Our Division has emphasized the need of having microscopic diagnoses on these cases, and in many areas nurses of medical dispensaries are paid for collection of slides from suspected individuals. The chief of the Division sent a letter to every one of these doctors and to those of Areas III and V, explaining the progress of malaria reduction and the importance of their cooperation in achieving eradication of the disease. In these areas it was emphasized, a case of malaria should be regarded with the same urgency as a case of plague or yellow fever, so that our Division could act quickly. Slides from these cases are sent to our field laboratories to be examined. They include both thick and thin films; the first is examined for 5 minutes. All of these slides, negative or positive for plasmodia, and all others collected by our personnel, are shipped to our Central Laboratory to be re-examined. All the alleged positive slides and not less than 10 per cent of the negative ones are again carefully searched. In this way it is believed that few if any mistakes are made in diagnosing malaria or in identifying parasites. Private or governmental clinical laboratories also report to our field personnel the positive slides which they find and make those slides available for confirmatory diagnosis. Finally, some cases go directly to our field laboratories to be examined. Progress made in Area I in microscopic diagnosis of suspected malaria cases is presented in Table 2, where in 1952 many more slides were examined than cases reported. This is because medical dispensaries located in traditionally malarious areas routinely take many more slides than the number of reported cases, as a last effort to establish the disappearance of malaria. Table 2

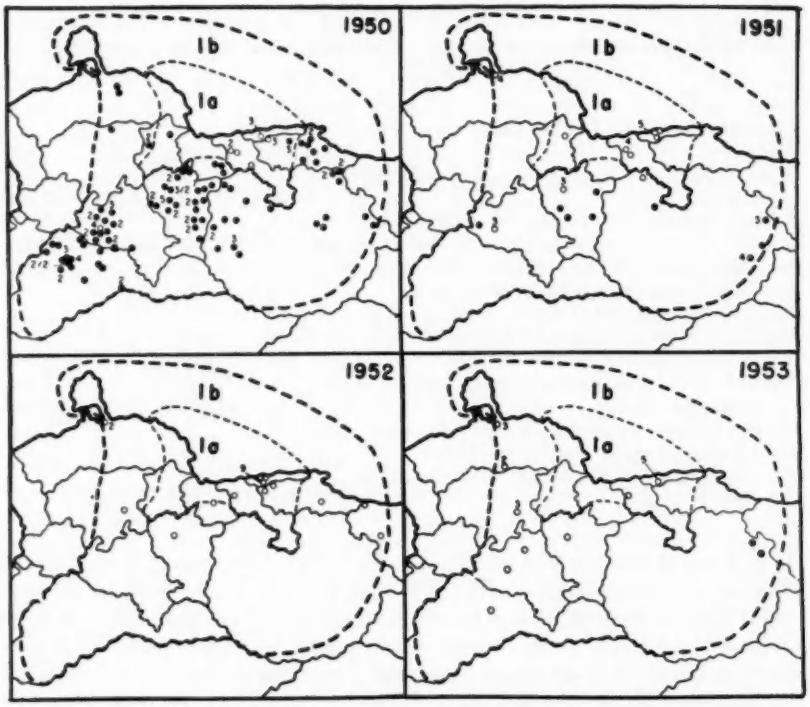

FIG. 2. Distribution of malaria cases in Areas Ia and Ib. Black dots represent indigenous cases and white dots represent imported cases. Figures at side of dots represent number of cases, and when there are two figures the first represents indigenous cases and the second, imported cases. When the dot has no figure it represents only one case.

also shows the great decrease in reported cases, and the very low percentage of positives found in the slides taken. This indicates that at present the number of reported cases exceeds the number of actual cases.

The activity of health visitors, who work mostly in rural districts, is shown by the large number of visits to houses that they made per year (Table 2). These men take a blood smear from every person found with fever at the time of the visit, or who report fever during the previous week. In their work they usually follow the itinerary of the spraying squads, so that most of the houses which are sprayed in the rural areas receive one or more visits per year. For instance, in 1952 in Area I 198,775 houses were sprayed of which 72,232 were in towns with doctors and 126,543 in rural districts without doctors. When physicians are present, we rely on information from medical dispensaries regarding malaria cases in that town, thus the activity of health visitors was mostly limited to the rural districts. The number of house visits they made this year was 163,253, or approximately 1.3 visits per house in the rural districts. All this indicates that the search

for malaria cases is a very exhaustive one. As seen in Table 2, the number of malaria cases found by this means is very small, indicating the virtual elimination of malaria from the area. It is interesting to observe in this table that the index of infection with malaria parasites (0.3 per cent) of the slides taken by the health visitors in 1952, was practically the same as the index (0.2) obtained the same year with the slides taken from reported cases. This shows the accuracy of the work, and that the results presented reflect actual field conditions.

The children examined splenometrically in Area I and those found with splenomegaly are shown also in Table 2. Among these, only one showed parasites, a sign that the disease is disappearing from Area I. This table also shows that the number of children with enlarged spleen is being reduced every year, a further indication of interruption of transmission.

Each case of microscopically diagnosed malaria is subjected to an inquiry, recorded on a form specially drafted to guide subprofessional personnel. Most of the microscopically diagnosed malaria cases in Area I, particularly in the last two years, have been personally questioned by the zone doctor.

In Area I, Area III north of Apure and Bolívar, and Area V, when a positive case is found in a locality where malaria transmission existed previously the "mopping up technique" is applied. This consists of an exploration by the inspector to study the presence and density of larval or adult stages of vectors, a survey by the health visitor in search of fever cases, to get slides from and give treatment to every suspected person, and a visit by a spraying squad to repeat treatment of all houses if more than three months have elapsed since the last spraying. These investigations cover an area of 5–10 kms. radius around the locality where the case was found. The zone doctors personally supervise this work. It is expected that with these measures an acceleration of malaria eradication from Venezuela will take place, though so far, no new cases connected with previous ones have been observed, a sign that probably most if not all the cases are relapses.

However, eradication of malaria from Area Ia was achieved without these special measures. As in Areas II and IV, wherever a large number of cases are found at present, due mostly to out-of-doors transmission, such measures are not applied. Also in Amazonas, Apure, Bolívar and Delta Amacuro, all of them in Area III, where distances from one locality to another are great, these measures are not used as they are not economically feasible. In this Area and in Area V, there are many places where not a single case of malaria has been observed for more than a year. These are separated from Area I by localities in which indigenous cases occurred in 1952. In that year these two areas had 20 per cent of the malaria cases diagnosed in the country.

Results of inquiries. Malaria cases are divided into *suspected* and *confirmed*, the last being the only ones considered here, as the microscope showed that many of the suspected cases did not have parasites. However, as infections of vanishing malaria have such low densities of parasites, it is impossible to be certain that all the unconfirmed cases were free from plasmodia. To eliminate this source of error has been out of the question. Every confirmed case in Area I, as in other

areas, is taken as an *indigenous case* unless the inquiry shows clear evidence to the contrary. So far no *introduced cases*, that is, autochthonous malaria contracted from imported cases, have been observed. For a person to be considered an *imported case* from another Area, he must have come from a district where indigenous cases are present or were present during the last year. One *induced case* was found in Area Ia due to an infection accidentally produced by blood transfusion. Imported cases found in Area I have been mostly relapses, but each relapse has been considered a separate case in the tables presented, since, being relapses from infections diagnosed outside Area I, their identification was not always possible with the degree of certainty required by the careful study to which every case was submitted. Indigenous cases have been so few and scattered that malaria in Area Ib in the last two years may be described as sporadic. Endemic malaria, therefore, has entirely disappeared from the whole of Area I.

Table 3 shows all malaria cases found in Area I, distributed by origin of infection and species of parasite. It may be seen that Area Ia, with the possible exception of four *vivax* infections found in 1950, has not shown indigenous cases in the last three years. One of these *vivax* infections appeared in February in Farriar, Yaracuy, and three were found in March in Araira, Miranda. The last cases known from these two municipios occurred in October 1948. In 1949 1670 house visits were made in Araira and 1851 in Farriar in search of cases, without obtaining any positive slides. There are doctors stationed in both municipios. On account of the absence of cases in 1949 in these two municipios, and due to the fact that the four infections mentioned above were found during the dry season, when transmission is greatly reduced, it seems reasonable and safe to consider these cases as relapses. Therefore, in Area Ia there have been no primary attacks of indigenous malaria in the three year period of 1950–1952. Furthermore, in the twelve months of 1953 no similar cases have been found in Area Ia. The rest of the cases of Area Ia had various origins. Four cases came from Area Ib, from localities where transmission occurred in 1950 and 1951. The largest number (13) came from Area IV, which is, as mentioned before, the most malarious area of Venezuela at the present time. Only 9 cases came from the other areas. Three cases came from abroad. Two cases were *vivax* relapses after a Cesarian and an abortion; these were found in Caracas where malaria has never been present. No data are available concerning the only other two cases of unknown origin found in Area Ia.

In 1950 and 1951 indigenous cases were found in Area Ib, but none in 1952, as shown in Table 3 and only two positives were reported in 1953, both of which occurred near the border. Of the cases imported from other areas, most came from Area IV, as happened in Area Ia. Only one case of unknown origin was found in 1952 in Area Ib. The cases whose origin is unknown in both areas were persons from whom slides were taken but could not be traced after it was known they had parasites. Their number in both areas, however, is negligible. Figure 2 shows every locality where cases of the years 1950, 1951, 1952 and 1953 were found, and gives an idea of their distribution and number.

Table 3 also indicates the distribution of cases according to parasite species. In

TABLE 3

Origin of malaria cases found in Area I

Origin	1950				1951				1952				1953			
	F	V	M	A*	F	V	M	A*	F	V	M	A*	F	V	M	A*
Cases of Area Ia																
From Area Ia	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—
From Area Ib	—	3	—	—	1	—	—	—	—	—	—	—	—	—	—	—
From Area II	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	—
From Area III	—	—	—	—	—	—	—	—	—	1	—	—	1	2	—	—
From Area IV	—	—	—	—	1	5	—	—	1	6	—	—	—	4	—	—
From Area V	—	1	—	—	—	3	—	—	—	1	—	—	—	1	—	—
Unknown or abroad	—	—	—	—	1	—	2	—	—	4	—	—	—	—	1	—
Total	—	8	—	—	3	8	2	—	1	14	—	—	1	7	1	—
Cases of Area Ib																
From Area Ia	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
From Area Ib	21	81	15	10	10	7	1	—	—	—	—	—	—	2	—	—
From Area II	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
From Area III	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—
From Area IV	—	1	—	—	1	2	—	—	—	5	—	—	—	6	—	—
From Area V	—	2	—	—	—	4	—	—	1	1	—	1	—	1	—	—
Unknown or abroad	3	2	—	—	—	—	—	—	1	—	—	—	2	—	—	—
Total	26	88	15	10	11	13	1	—	2	6	—	1	2	9	—	—

* Association of *vivax* and *falciparum*.

Area Ia no double infections were found in the last three years, and the only one observed in 1952 in Area Ib came from a locality of Area V where transmission still occurs. The disappearance of mixed infections is a clear sign of reduction of transmission and of low density of parasites. *P. malariae* was not found in either Area during the years under consideration, with the exception of one infection which came from abroad, and another of unknown origin which appeared in a locality where malaria had been absent during the last three years. *P. falciparum* decreased markedly in Area Ia in 1950–1952 and in Area 1b in 1952. The only case

TABLE 4

Malaria cases found in Area I according to the month in which they were discovered

Months	1950		1951		1952		1953	
	Ia	Ib	Ia	Ib	Ia	Ib	Ia	Ib
January	1	21	—	3	2	—	—	—
February	1	17	1	4	—	—	—	5
March	3	21	1	—	1	—	2	—
April	—	11	—	4	2	2	2	1
May	—	10	2	3	1	1	—	1
June	—	13	—	2	1	2	1	1
July	—	13	3	2	2	2	—	—
August	—	5	—	2	3	1	—	2
September	2	13	—	—	—	—	1	—
October	—	3	2	2	2	—	3	—
November	—	10	3	2	1	—	—	—
December	1	2	1	1	—	1	—	1
Total cases	8	139	13	25	15	9	9	11
Number of slides examined	2925	6423	2310	5815	2505	6967	2160	8401
Percentage of infection	0.3	2.2	0.6	0.4	0.6	0.1	0.4	0.1

with this species whose origin could not be ascertained was found in a locality where no positive slides had been observed in the last three years. *P. vivax* has been the most prevalent species in the areas and years under discussion. Of these infections, one came from abroad, two were relapses after the Cesarian and abortion mentioned above, and one was a case about which no clear data could be obtained.

As mentioned above, some of the cases presented in Table 3 were confirmed relapses, all of *vivax* infections. Two of these were observed in Area Ia, which reduces to 7 in 1951 and to 13 in 1952 the number of *vivax* infections observed in this area. In Area Ib five relapses were identified in 1950, one in 1951 and two in 1952, which reduced accordingly the number of infections reported in Table 3 from this area.

The monthly incidence of cases in Area I is recorded in Table 4. In Venezuela malaria is more prevalent in the last six months of the year. In Area Ia during the three years under consideration there were generally more cases in this period, as was to be expected. In 1950 in Area Ib the number of cases observed in the second six months of the year (46) was almost half that found during the first six months (93). This is due to the continuously decreasing transmission of malaria following DDT spraying in this area. As some transmission still occurred the following year a similar situation was found in 1951, 16 cases in the first six months and 9 in the last six months. In 1952, when no indigenous case was observed in Area Ib, the number of cases in both periods was about the same, 5 and 4 respectively.

Table 4 also shows that the percentage of slides with malaria parasites in-

TABLE 5

Malaria cases found in Area I according to age groups

Age Groups, Years	1950		1951		1952		1953	
	Ia	Ib	Ia	Ib	Ia	Ib	Ia	Ib
1	—	4	—	—	—	—	—	—
1–4	1	21	—	3	—	—	—	1
5–9	—	17	1	4	1	—	1	1
10–19	—	24	—	4	1	—	—	2
20–29	1	27	1	6	5	2	5	5
30–39	2	7	—	3	5	5	2	2
40–49	—	10	1	1	2	1	—	—
50–59	—	1	—	2	—	—	—	—
60–69	—	0	3	—	1	—	—	—
70+	—	0	—	—	—	—	—	—
Unknown	4	28	7	2	—	1	1	—
Total.....	8	139	13	25	15	9	9	11

creased in Area Ia from 0.3 in 1950 to 0.6 in 1952; on the other hand, in Area Ib this percentage decreased from 2.2 in 1950 to 0.1 in 1952. The increase of cases observed in Area Ia is probably due to the arrival of more people from other areas than occurred in Area Ib; to having better medical facilities than Area Ib on account of the large number and concentration of doctors so that it was easier to find the cases; and to being smaller than Area Ib so that the search for cases was more accurate.

Table 5 shows the age distribution of the malaria cases found in Area I. In Venezuela, in spite of the fact that malaria mortality is highest below one year of age, malaria morbidity is highest in the groups 1–4 and 5–9 years. In Area Ia most of the cases found in the period 1950–1952 were 20 years old or more, which is explained by the fact that they were imported cases. In Area Ib in 1950 there was a large number of cases in the group 1–9 years since transmission was still occurring but later, as soon as transmission disappeared, most of the cases were in the group above 20 years, for the reason just mentioned. Age distribution, therefore, confirms the elimination of malaria from Area I.

It would seem, therefore, after careful consideration of the above data, and discussion of the cases of malaria found in Area I, that even the most sceptical mind will agree with the conclusion we have reached, that malaria has been eradicated from a large zone of Venezuela. The epidemiological work which backs that conclusion has not been easy, but we believe that it may be compared favorably with similar investigations carried out in the more developed countries of the temperate zone. It is expected that the future will confirm the results presented here. No failures of magnitude are expected, as it will be practically impossible, with the state of mind which has been built up in the medical profession and the lay people with reference to the malaria problem, that an outbreak would occur without receiving prompt attention. The experience obtained in Area I

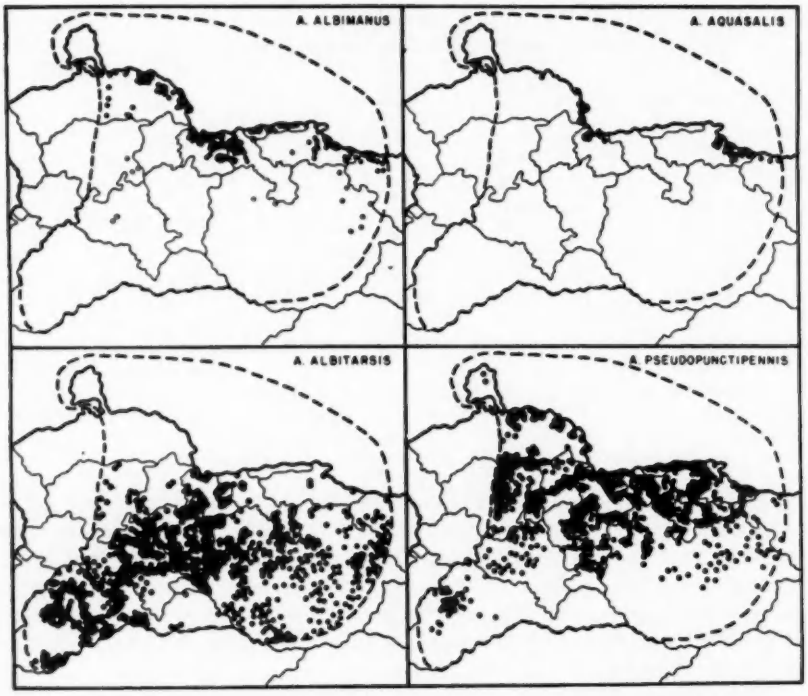

FIG. 3. Geographical distribution of the four vectors present in Area I

clearly indicates that eradication of malaria from the whole of Venezuela can be reached and has to be reached.

VECTORS AND MALARIA ERADICATION

The geographic distribution of the four incriminated vectors present in Area I is shown in Fig. 3. Of these, the only one of real importance in Area I is *A. albimanus*, which occurs in the northern portions with occasional appearances in some localities of the interior. This mosquito was a vector in the Costa-Cordillera region and did not play any role in the Llanos region, from which it appeared and disappeared spontaneously. The population of these four species has not been affected by DDT spraying (Gabaldon, 1952).

On the other hand, *A. darlingi*, the most important vector in Area I, has been drastically reduced by DDT spraying. This species, as shown in Fig. 4, had a wide distribution before DDT was applied and, as it is a highly anthropophilic indoor-biter and house-haunting mosquito, it has practically disappeared from the area. In some States, as shown in Table 6, the species may be considered to be eradicated, since it has been absent from house captures for several years. No larvae of this mosquito have been found in Area I since 1949, although from 5,000

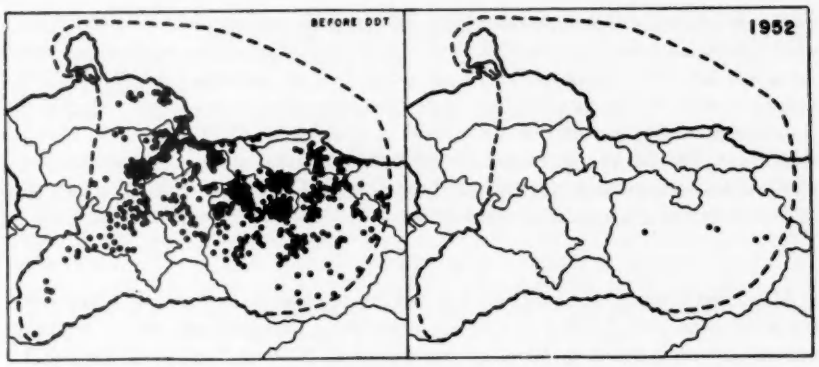

FIG. 4. Distribution of *A. darlingi* in Area I to show progress in its eradication

TABLE 6

Indices of infestation (houses) with A. darlingi *in Area I to show development of eradication of the species*

States	1941–1945	1946	1947	1948	1949	1950	1951	1952	1953
Anzoátegui	8.2	2.0	—	0	0	0	0	0	0
Aragua	1.3	0.5	1.3	1.2	0.3	0.0	0	0	0
Barinas	0	—	0	1.2	0.2	0.2	0	0	0
Carabobo	6.4	2.1	0.1	0	0	0	0	0	0
Cojedes	33.2	9.4	1.7	1.0	2.7	0.3	0	0	0
Distrito Federal	1.7	0	0	0	0	0	0	0	0
Falcón	2.4	8.3	8.2	3.1	0	0	0	0	0
Guárico	4.9	9.1	0.6	0.5	1.5	0.4	0.1	0.2	0.0
Lara	0.5	1.2	0.1	0	0.0	0	0	0	0
Miranda	0.1	1.2	2.7	0.2	0.5	0	0	0	0
Portuguesa	17.8	5.2	0.9	0.8	0.5	0	0	0	0
Yaracuy	4.0	5.3	1.5	0.0	0	0	0	0	0
Total number of capture stations examined	81,008	36,101	46,566	58,491	60,195	54,465	72,308	67,478	82,381

to 6,000 breeding-places were visited each year for the next three years. The density of this anopheline was always higher in houses than in breeding places. All available experience indicates that *A. darlingi* will be entirely eradicated from Area I in the near future.

The elimination of *A. darlingi* from Area I is a factor to be considered in connection with the eradication of malaria from this area. It seems logical to think that with the extermination of the most important vector malaria transmission would be entirely interrupted. Thus, malaria eradication might be a natural consequence of the elimination of this vector. But that was not the case in Area I. At least two of the other vectors present, *A. albimanus* in the North and *A.*

albitarsis in the Southwest, were responsible in some localities for a high incidence of the disease in the absence of *A. darlingi*. Therefore, malaria eradication from Area I is not only the result of the elimination of *A. darlingi* from most of the area, but also of the interruption of transmission due to other vectors, whose population was apparently not modified in density by DDT spraying. This is important, because it clearly indicates that in a tropical zone, in the presence of efficient vectors, malaria may be eradicated by DDT indoor residual spraying, and that vector eradication is not necessary to obtain malaria eradication.

SUMMARY

After eight years of a nation-wide campaign against malaria in Venezuela, malaria has been eradicated over a large area. The criterion of cessation of malaria endemicity established in 1950 by the National Malaria Society of the U.S.A. has been adopted. This area, where almost half (49 per cent) of the population of Venezuela lives, is believed to be the largest area, within the tropical zone, of malaria eradication following residual insecticide spraying.

In this area of North-central Venezuela, within the Costa-Cordillera and Llanos geographic regions of the country, the intensity of malaria was as high as in the most malarious zones of tropical America. The incriminated vectors have been *A. darlingi*, the most important of the Neotropic Region, and *A. albimanus* and *A. albitarsis*. Highly endemic and epidemic malaria were found in sections of the area.

DDT, applied at the rate of 2 gm. per sq. meter every six months, has been the insecticide used. At the beginning, however, 1 gm. every three months and later every four months was employed. In some districts, after malaria transmission ceased, other insecticides have been utilized: chlordane against flies and BHC and dieldrin against triatomids.

After exhaustive epidemiological investigations, which may be compared favorably with some used in the more developed countries of the temperate zone, the following facts were established. In one area of about 20,000 sq. km. no primary indigenous case of malaria has been discovered during the last three years. In another area of about 160,000 sq. km. no primary indigenous case of malaria was observed, during the last two years, with the exception of 2 cases near the border of the area, which seems to indicate that here also malaria endemicity has entirely ceased.

Of the incriminated vectors, *A. darlingi* has been eliminated by DDT residual spraying and is apparently eradicated from most of the territory under discussion. But the population density of the other two vectors, *A. albitarsis* and *A. albimanus*, has not been affected by the insecticide. This indicates that in a tropical zone in the presence of efficient vectors, malaria may be eradicated by DDT residual spraying without vector eradication.

With this experience it is expected that malaria eradication from most of Venezuela will be attained in the near future. Only two exceptions to this generalization are considered probable. The first involves two small areas representing only 3.4 per cent of the territory previously malarious, where out-of-doors transmission

is taking place which will require additional measures. The second includes those districts inhabited by some nomadic and rather wild Indian tribes, most of them in Amazonas, Apure, Bolívar and Delta Amacuro, which may have to wait for their incorporation to Venezuelan society before they can be freed from malaria.

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REAPPEARANCE OF *ANOPHELES DARLINGI* ROOT IN A CONTROLLED AREA OF BRITISH GUIANA'S COASTLANDS

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The eradication of *Anopheles darlingi* and its concomitant severe endemic malaria from the coastlands of British Guiana, solely by residual DDT techniques, was reviewed by the senior author in 1948 and 1951, and is being fully maintained at the time of writing in the sixth year after the establishment of a comprehensive control program. The temporary reappearance of *A. darlingi* in a restricted section of the Colony's eastern coastlands, and its association with a newly-constructed drainage-irrigation project, forms the subject of this report. This episode detracts in no way from the published results; in fact, it illustrates some important features in the biology and control technique against this dangerous, widely distributed and most important vector species of Tropical America.

INTRODUCTION

The coastal belt of British Guiana comprises a narrow alluvial fringe 5 to 10 miles deep extending for 280 miles along the sea coast and the banks of the larger river estuaries. This narrow strip sustains approximately 90 per cent of the Colony's 435,000 inhabitants, engaged primarily in the cultivation of sugar cane and rice. Because the area is largely below high tide level, an intricate system of sea defences, sluice gates, drainage and irrigation canals and pumping stations constitutes a common and essential feature of local agricultural techniques and plantation and village topography. Prior to the DDT era, this complex hydrological system and the cultivation of the two staple crops supplied all the conditions required for the abundant and continuous production of *A. darlingi* in the immediate surroundings of towns and villages. Indeed, malariometric rates were usually directly proportional to the degree of development of drainage and irrigation works, and therefore, to the stage of agricultural advancement in each district (Giglioli, 1938; 1939; 1948).

In the eastern part of this coastal belt, on the Corentyne Coast, agricultural development was less advanced, and the man-made factors which provided the conditions for coastal malaria endemicity were relatively deficient in extent and restricted in their distribution. Here, the villages are mostly surrounded by extensive brackish and wind-swept marshes which are unsuitable for *A. darlingi* production. In the past, malaria manifested itself in epidemic form only during years of exceptionally high and prolonged rainfall, when changed ecological conditions favored temporary invasion of this mosquito vector from its endemic strongholds in the irrigated lands on the estuaries of the Berbice and Corentyne

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Rivers. The last such invasion and resultant malaria epidemic in this area was observed during the heavy rains of 1938, and ended with the severe drought of 1939–1940. Between such rare epidemic waves, low endemicity prevailed (spleen rates below 10 per cent), largely maintained by infection contracted outside the district.

DDT control was initiated experimentally in the Colony in February, 1945; by 1947 the work was proceeding on a Colony-wide scale. The Corentyne Coast, however, was one of the last areas to be tackled, and an initial application was made at the southern and northern extremities of this area in 1948. The central section was subjected to a single application in 1949 primarily for the eradication of *Aedes aegypti*. Whereas, however, repeat applications were made at the southern end (along the Corentyne River estuary) in 1949 and at regular intervals thereafter, DDT spraying was cut back at the northern end as shown in Fig. 1. The concept of malaria control by "strategic barrier" technique (Giglioli, 1951) therefore found its first practical application in this district, and indeed residual DDT spraying was not repeated here except in the south over a 10-mile expanse of populated coastland on the left bank of the Corentyne estuary; and in the north over a 7-mile coastal stretch approaching the right bank of the Berbice River estuary (Fig. 1). It was in the southern half of the gap between these two zones of permanent "barrier" control that *A. darlingi* was discovered in the course of routine mosquito surveys in October-November 1951.

As reported by the senior author (Giglioli, 1951) the "strategic" use of DDT for the maintenance of malaria control has given completely satisfactory results in British Guiana; routine post-DDT mosquito surveys of the coastlands, including part of the areas under review, were negative for both *A. darlingi* and *Ae. aegypti*. During the period December, 1949, to June, 1950, a large section of the Corentyne Coast was inspected only for *Ae. aegypti* breeding foci and results were negative. No systematic adult mosquito house captures were made, however, so that no definite data on the *A. darlingi* situation were procured for that period.

Field experiments and overall experience have shown that the residual action of DDT under local housing, climatic and social conditions is appreciably more durable than that originally anticipated. Thus the interval between spraying cycles was progressively increased from 6 months in 1945 to 18 months in 1950. That this procedure in no way reduced the efficiency of the program is illustrated by the fact that the number of malaria cases reported on the Corentyne Coast (population 56,400), and based almost exclusively on clinical examination, fell from 656 in 1949 to 15 in 1952. Epidemiological investigtion of 3 positive cases reported in 1951 established without a doubt that these infections had been contracted on the Canje Creek and not in the coastal villages. This general picture of actual malaria eradication is now common to the entire coastlands of British Guiana where the total number of unconfirmed cases returned annually has fallen from 7,411 in 1949 to 41 in 1952 (B. G., 1952).

Economies in labor and materiel effected in this control program, first by extension of the interval between spraying cycles and later by adoption of the "barrier" technique, permitted the application of equally satisfactory control

measures to the small and widely scattered settlements on the minor rivers and creeks of the coastal hinterland. One such stream, the Canje Creek, running parallel with the Corentyne Coast and only 12 miles southwest of the settlements which border the public road (Fig. 1), was first surveyed and brought under control in February, 1951. Here *A. darlingi* was found in large numbers, with morning hand-captures averaging 49.6 per house; and spleen and parasite rates of 51.5 and 13.7 per cent respectively were recorded among 101 children (B. G., 1951). The presence of *A. darlingi* in this riverain area at this late date in British Guiana's control program was due mainly to transportation difficulties in reaching the locality, and was the primary cause of its break-through to the Corentyne Coast in 1951. This break-through substantiated our own contention (Giglioli, 1948; 1951) that human intervention and traffic may be expected to promote *A. darlingi* invasion of hitherto free areas, and Bruyning (1952) has since made a similar prediction in Surinam. Furthermore, it proved that two tactical errors were made in the formulation of the strategic plan, namely (a) the upper Canje Creek was not included *ab initio* in the area of permanent control; (b) the construction of a new hydraulic scheme connecting the Canje Creek with the inhabited coastlands was overlooked during its early stages.

THE AREA OF *A. DARLINGI* REINFESTATION

The eastern half of the Corentyne Coast presents a striking example of ribbon development, with villages and settlements adhering closely to the public highway. The houses are mostly 2 to 4-room wooden cottages, usually unpainted; some are constructed with poorer material such as split manicole palm or mud and wattle. Such mud-walled structures here, however, comprise only a small proportion of the total houses, and have not, therefore, presented a problem in the local DDT campaign to the same degree as experienced in other territories. Nevertheless weekly "re-daubing" is a common practice, and during certain religious festive seasons very thorough house-cleaning is carried out with vigorous scrubbing of the inner surfaces from floor to plate.

Peasant lands, chiefly under rice cultivation and pasturage, seldom extend more than 3 miles inland in the section, but as part of the Colonial Government's comprehensive drainage and irrigation development program, a water-control scheme for the benefit of this zone has been under construction since 1949. This scheme (designated Block III) provides for bonification of 48,000 acres and is based on pumping water from the Canje Creek to high-level irrigation canals which are associated with a net-work of low-level drainage trenches with main outfalls at Nos. 51 and 66 Villages (Fig. 2). These canals and drains are employed as internal transportation waterways. The lands within the scheme comprise open savannah with a few more elevated sandy reefs covered with light forest; the soil is predominantly heavy clay, bearing approximately 2 to 3 inches of acid pegasse topsoil. At its eastern perimeter the Block III scheme embraces 24 villages.

Operations were commenced in 1949 and were completed in 1951. Two floating draglines were used which carried sleeping accommodation for 30 workmen;

TABLE 1

Monthly rainfall (inches) at Pln. Skeldon, Corentyne Coast, British Guiana, 1938 and 1949–1951

Years	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Annual Total
1938	14.50	18.49	8.43	15.15	10.89	10.99	6.56	7.51	2.95	1.75	0.71	5.54	103.47
1949	11.21	8.80	5.82	10.00	9.77	7.74	5.01	6.62	3.72	2.81	4.71	10.68	86.89
1950	14.47	1.78	2.41	1.79	10.66	12.24	7.31	5.39	0.99	1.73	6.30	6.27	72.84
1951	17.70	6.70	2.37	5.25	11.31	14.37	7.00	5.88	0.08	2.76	0.14	6.73	80.27

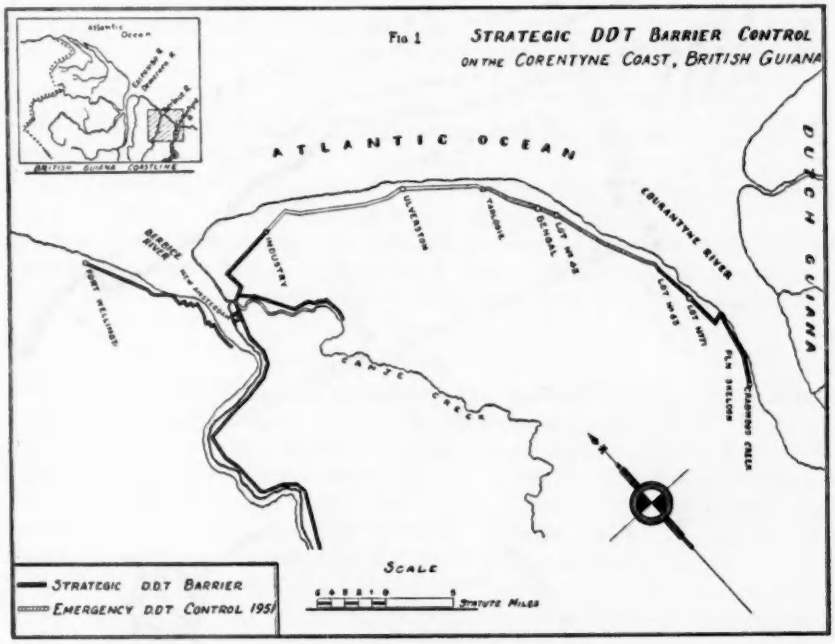

FIG. 1

temporary camps were built along the water-path as the work progressed for a labor force of approximately 120; and two permanent watchhouses were erected. A number of burrowpits left unfilled and undrained after completion of the project developed into ideal mosquito production foci.

Representative rainfall data for the area under study are shown in Table 1, as recorded during 1949–1951, on a sugar estate on the Corentyne estuary situated approximately 6 miles to the south of the area (Fig. 1). The mean annual total of 80 inches for those three years approximates average normal conditions. The unusually heavy precipitation of 103 inches recorded in 1938 has been in-

cluded in Table 1 firstly to emphasise the close correlation between the abnormal rainfall of that year and the appearance of *A. darlingi* and epidemic malaria on the Corentyne Coast as referred to above, and secondly to illustrate the absence of any such natural phenomenon with this mosquito's reappearance here in 1951.

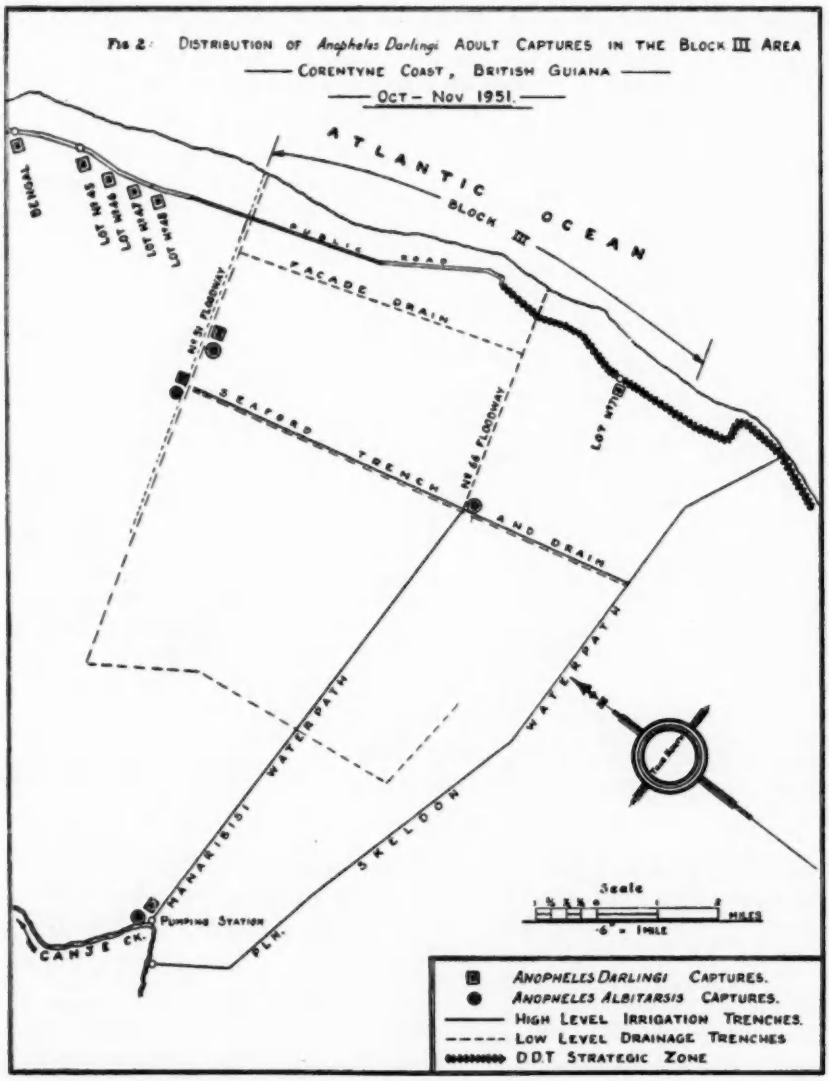

FIG. 2

TABLE 2

Houses positive for A. darlingi *adults, Corentyne Coast, British Guiana, 1951*

Status of Houses in Relation to Last DDT Spraying Cycle	Number	Per Cent
New houses: erected after last DDT cycle	6	31.5
Old houses: interior walls altered—redaubed	4	58.0
repapered	3	
scrubbed	2	
painted	1	
reconstructed	1	
interior walls not altered	2	10.5
Total	19	100.0

Thus, the principal changes in local ecology brought about by the Block III drainage scheme were:

(A) *Hydrological:* Construction of canals and trenches; introduction of water from the Canje Creek; the excavation of numerous burrowpits.

(B) *Human:* House boats, temporary and permanent camps and watch houses providing a permanent, though shifting human population in the area and a continuous stream of human traffic between the Canje and the coastal settlements.

ENTOMOLOGICAL FINDINGS

In the course of routine mosquito surveys in October-November, 1951, 25 female adult *A. darlingi* were collected from 20 houses over a 24-mile stretch of the Corentyne road. The total number of occupied houses in that area was 5,067, 41.8 per cent of which were examined at random. All but one of the positive houses, with one *A. darlingi* adult, were situated within a 14-mile section of the roadway immediately east and north of the Block III perimeter where 76.6 per cent of a total 1,444 houses were inspected. Of the 19 positive houses here, only 5 were within the "strategic" zone of repeated DDT applications (Fig. 2)—the most recent spraying having been made 12 months previously. The last positive house was situated at Ulverston, 13 miles beyond the northern extremity of the area under discussion.

Detailed investigations in relation to the last DDT spraying were made on these 19 positive houses. In all but two of them (10.5 per cent), such extensive alterations to their interior had been carried out as to reduce or eliminate the effectiveness of that DDT application (Table 2). Simultaneous larval searches in the adjoining villages yielded no *A. darlingi*. Captures made within the Block III area yielded 95 adult *A. darlingi* during a single inspection: in 3 trash houses near the pumping station; at the No. 51 watch house on the main floodway drain to the north; and at an open temporary shelter nearby (Fig. 2). Fifty house-caught adult *A. albitarsis* were also collected in the area, but limited larval searches again proved negative for *A. darlingi*.

CONTROL MEASURES

Because of these entomological findings, an emergency residual DDT program was instituted embracing 1,469 premises in that part of the normally unsprayed section of coast in which *A. darlingi* was encountered. In view of the indications of gradual dissemination northwards from Block III, this program was extended 8 miles beyond the last positive village. In addition, watch houses, other premises, camps and dragline-huts within the drainage area were also treated, and the spraying schedule for the Corentyne estuary "barrier" zone was advanced so that by January, 1952, the whole affected locality had been brought under control. No control measures were instituted at Ulverston where a solitary *A. darlingi* was captured, and two subsequent mosquito check surveys at 3-month intervals after January, 1952, failed to reveal further presence of *A. darlingi* in the area under review, including Ulverston and its environs.

DISCUSSION

In 1944 before any literature on DDT reached the Colony, plans for increased agricultural bonification of the coastlands in general were drawn up by the Colonial Government, which included the erection of sea-defences and the introduction of drainage and irrigation schemes on the Corentyne Coast. The Block III scheme described above represents only part of these original plans. Even before the introduction of DDT control, the Corentyne Coast, with its low malaria prevalence, was mainly responsible for maintaining the Colony's vital index. When, therefore, these large-scale engineering projects were made known in 1944, one of us (G. G.), in an unpublished departmental report, documented the close relationship existing between severe endemic malaria on the coastlands and local agricultural bonification schemes (see also Giglioli, 1946). It was predicted that the introduction of such drainage and irrigation schemes on the Corentyne Coast would undoubtedly bring about the introduction and establishment of *A. darlingi* and hyperendemic malaria. It may therefore be considered fortunate that lack of adequate development capital during this period resulted in postponement of these agricultural schemes until the postwar period when DDT became available.

There is no doubt that with the highly selective and domestic habits of *A. darlingi* in British Guiana, DDT has resulted in the eradication from the coastlands of both this mosquito and malaria. The episode we have recorded indicates how well-founded was the forecast of 1944, for, without this insecticide, *A. darlingi* and endemic malaria would in all probability have been firmly entrenched on the Corentyne Coast today, following in the wake of operations on the Block III scheme.

Although *A. darlingi* adults were not captured in the two draglines at the time of the investigations, the role which their mobile crews' quarters, occupied temporary camps, and burrowpits all played in *A. darlingi*'s penetration of the 12-mile gap of hitherto uninhabited lands between the Canje Creek and the coastal villages, is well illustrated in Fig. 2 which shows that specimens were collected at

the two extreme ends of the water control project. The discovery of *A. darlingi* adults (at low density) in houses along the public road was apparently made in the early phases of its dissemination, for it must be emphasised that the 19 positive houses encountered in the Block III perimeter constituted only 1.7 per cent of the total number inspected within the area of actual reinfestation. It was also of interest to note that this dissemination occurred predominantly in a northerly direction, away from the "strategic" DDT control barrier (Fig. 2). Because of this, it was concluded that the isolated specimen collected 13 miles to the north of the reinfested area was accidentally transported by some motor vehicle—especially when it is borne in mind that even in the absence of any control measures, subsequent mosquito house captures failed to yield additional *A. darlingi* adults in that locality.

The entire incident brought home forcibly to us (a) the undoubted value of continuous entomological check surveys as an integral part of any control program; and (b) the absolute necessity for close collaboration between the Department of Public Health and other Government Departments or industrial organisations undertaking large-scale development projects in the backlands of the Colony. It may perhaps be reiterated here, that apart from suggesting these valuable guides to future control policy, this occurrence of *A. darlingi* reinfestation has not been permitted to curtail development of the "strategic barrier" technique in British Guiana. On the contrary, a further revision of the local control program involving further cutback of the "barriers" illustrated in Fig. 1 has been put into force at the time of writing in 1953.

SUMMARY

On the greater part of the coast of British Guiana, close association has hitherto existed between hydrological bonification and hyperendemic malaria, except on the eastern coastlands. Residual DDT house spraying, as the sole means of control, brought about the elimination of *A. darlingi* and *Ae. aegypti*, and an economic technique of control maintenance by "strategic barrier" spraying was gradually developed.

In 1951, *A. darlingi* reappeared suddenly in small numbers on the eastern coastlands in the wake of artificial changes in conditions of physical and human ecology in a localised area. Prompt spraying of houses in the villages and along the path of invasion eliminated a potentially dangerous threat from *A. darlingi*-borne malaria in the district. This incident confirmed rather than weakened the principle of "strategic barrier" spraying in British Guiana.

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THE ROLE OF INSECTICIDES IN THE TVA MALARIA CONTROL PROGRAM

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From the beginning, the TVA malaria control program has emphasized measures directed at control of the vector, *Anopheles quadrimaculatus* Say. Naturalistic or environmental control measures have been preferred to the use of insecticides. The objective of program planning has been to maintain a balance among the various control measures available so as to achieve effective mosquito control as economically as possible.

Principal elements of the control program are water level management, permanent shoreline improvement, shoreline and drainage maintenance, aquatic plant control, and larviciding. Limited use was made of mosquito-proofing (Watson and Maher, 1941; Krusé and Gartrell, 1945) and DDT premises spraying (Hess and Keener, 1946; Hinman and Cutkomp, 1947) pending the establishment of satisfactory larval control measures. In a section of one reservoir, special malaria control easements were purchased which prohibited nighttime occupancy in the one-mile zone during the anopheline breeding season.

Water level management is the primary control measure now being utilized. It adversely affects the vector and restricts the invasion of the pool by the marginal vegetation in which the anopheline larvae develop. Permanent shoreline improvement (Gartrell and Kiker, 1948), plant growth removal and control operations, and marginal drainage maintenance serve to increase the effectiveness of the water level management program. Larvicidal operations which, at present, consist of the application of DDT sprays by aircraft are depended upon: (1) to permit wider latitude in utilizing water level management for control of marginal vegetation, (2) to provide larval control during periods of unfavorable water schedules, and (3) to supplement other mosquito control measures in situations where they do not provide the desired degree of control. The over-all economy of the malaria control program depends in large measure upon the availability of an effective larvicide which can be easily and economically applied.

TVA experience in the use of DDT. Following exhaustive tests from 1947–1949 by Cutkomp *et al.* (1950) and Krusé *et al.* (1949, 1951), the aerosol equipment on the planes was replaced with spray equipment. Two fixed-wing aircraft and two helicopters were used in larviciding operations this past season. When DDT was distributed from aerosol generators, it was necessary to apply 0.1 lb. per acre due to the production of large numbers of small spray droplets which drifted away from the breeding area. When the change to sprays was made, the rate of application was reduced to 0.05 lb. per acre due to the higher rate of deposition obtained. Subsequently, it became necessary to increase the rate of application as a result of a change in larval susceptibility to this chemical (Krusé *et al.*, 1952).

Application rates of 0.1 lb. per acre and 0.3 lb. per acre are currently being used, depending upon the amount of vegetative cover over the area being treated.

There have been trends toward the abandonment of environmental control measures because of the spectacular results obtained through the use of residual applications of DDT, etc. for malaria control. However, in 1949, TVA embarked on a major construction program to eliminate by cut and fill operations all major anopheline breeding areas on its reservoirs. The total program proposed, involving approximately 11,000 acres of mosquito breeding area, is approximately 70 per cent complete. Progress on this program and cumulative beneficial effects of level management upon marginal plants have been effective in reducing larvicidal requirements. For example, in fiscal year 1938, Wheeler Reservoir (1,063 miles of shoreline) required approximately 95,000 acres of airplane dusting (with Paris green) and 4,800 miles of boat and hand oiling. In fiscal year 1953, only 4,600 acres of airplane larviciding were required in this reservoir. Larvicidal operations are now routinely required on only six of the twenty-five reservoirs. Upon completion of this permanent shoreline improvement program, larvicidal requirements will be reduced to a minimum.

During the period 1945–1950, DDT residual spraying of premises was employed in limited portions of three of the reservoirs. This measure was applied in areas where mosquito-proofing of dwellings had been employed in prior years in lieu of larviciding.

DDT resistance studies. Observations made by Ludvik *et al.* (1951) during the summer of 1950 pointed to the possibility that *A. quadrimaculatus* in the TVA reservoir areas might be becoming resistant to DDT. A comprehensive investigation of this possibility was begun in 1951. In addition, an analysis of data from annual bioassay and calibration tests of larvicidal aircraft for the period 1944–1951 was made. From this, it appears the the susceptibility of *A. quadrimaculatus* larvae to DDT remained unchanged until 1950. That year the recovered amount of spray necessary to obtain 50 per cent mortality was about ten times that required in 1949. In 1951, approximately a 25-fold increase over the amount for 1949 was required to reach the LD_{50} level. The amounts of DDT required to give 90 per cent mortality through the years are presented in Table 1, which would indicate that an even greater degree of change in susceptibility has occurred.

Field tests carried out against natural populations of mosquitoes in 1951 showed that it was necessary to double the rate of application (0.05 lb./acre to 0.1 lb./acre) of DDT larvicide in order to obtain results as satisfactory as had

TABLE 1

Recovery required in lbs. DDT/acre for 90 per cent kill of insectary-reared 4th instar Anopheles quadrimaculatus

Year	DDT/Acre Lbs.	Ratio to 1944
1944	.0005	1
1946	.0021	4
1949	.0019	3.8
1950	.0200	40.0
1951	.1300	260.0

been obtained during previous years. This higher rate has continued to give satisfactory results during 1952 and 1953. It may be that the increased use of chlorinated hydrocarbon insecticides for boll weevil control in cotton fields adjacent to mosquito breeding areas has been a factor in changing the susceptibility of *A. quadrimaculatus* to DDT.

Laboratory tests with larvae and adults from areas receiving DDT treatment and from untreated areas failed to show significant differences in susceptibility. Chemical analyses for *p,p'*-DDT and a metabolite, *p,p'*-DDE, made with adults and larvae from these two situations do show, however, that this species is capable of producing varying amounts of the metabolite. The examination of larvae has been complicated by the fact that no precise method for the direct application of DDT solutions has been developed and that physiologically active *p,p'*-DDT suspensions are physically unstable and give erratic mortality and analytical data.

A method for treating adults topically has been developed by Ludvik (1953), but individual susceptibility is very high and large numbers must be treated to obtain sufficient *p,p'*-DDT for chemical analysis. Though no clear distinction has been demonstrated in the differential production of *p,p'*-DDE from adults from treated and untreated areas, consistent differences have been noted in the total recovery of the applied doses, total recovery always being less in adults from treated than in those from untreated areas.

Efforts have been made to establish an insectary colony of *A. quadrimaculatus* resistant to DDT through topical application of measured amounts of DDT solutions to virgin adults. The survivors have been allowed to mate and the progeny reared and treated. This colony has reached the eighteenth filial generation. Dosage mortality data compared with data obtained from tests with insectary adults made in 1951 show that there has been little change in the level of susceptibility. It would be desirable in this effort to treat larvae instead of adults, since our control program is directed primarily against the larvae.

Ludvik *et al.* (1953) have compared observations made in 1944, 1950, 1952, and 1953 and shown that DDT has lost a great deal of its effectiveness as a residual insecticide for *A. quadrimaculatus*. There has been a marked decrease in the rate of knockdown and mortality of adults released in, and confined to, treated rooms. In 1944, a 200–250 mg. per square foot deposit produced 100 per cent knockdown and mortality in one and a half hours. In 1950, a similar application produced a 75 per cent knockdown only after 4½ hours ,with 100 per cent knockdown resulting within 16½ hours, and a 24-hour mortality of only 85 per cent being obtained. When adults were given opportunities to leave treated rooms *via* exit traps, 50–60 per cent did so in 1944, whereas 75–85 per cent of groups from treated areas left in 1950–53. Fifty per cent of the adults from an untreated area left the room in a 1950 test. In 1944, 100 per cent mortality in such tests usually occurred within four to six hours, but on one occasion it was only 95 per cent after four hours, and on another, 99.4 per cent after 38 hours. In 1950, 1952, and 1953, 24-hour mortalities ranged from 25 to 55 per cent in adults from treated areas. A group from an untreated area gave a 65 per cent 24-hour mortality in 1950. The rate and duration of exodus were always lower and shorter and the rate

of knockdown within the room much higher in adults from untreated areas. The age of the residual deposits in the tests cited was about six to eight weeks for all years.

SUMMARY

TVA has used DDT extensively as a larvicide since 1945 and to a lesser extent as an adulticide during the period 1945–1950. It is still effective for use as a larvicide for control of *A. quadrimaculatus* on TVA lakes. Its use as a residual spray has been discontinued, and its effectiveness for this purpose is now highly questionable.

In the TVA control program, chemical control measures have always been considered as supplementary, principal reliance being placed on environmental or naturalistic control methods. However, insecticides have been invaluable in the development of the program. They have served well pending the establishment of satisfactory environmental controls. There is a continuing need for efficient larvicidal measures for use when emergency situations arise and as a part of the regular control program to obtain the maximum benefit from water level management schedules. All control measures are frequently subjected to critical evaluation as to their economy and effectiveness under current conditions and changes made as might be indicated. DDT larvicidal measures are under close scrutiny at present because of the possibility of development of resistance. Concurrently, investigations of new insecticidal materials are underway so that a satisfactory substitute for the DDT larvicide will be available if needed.

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SUSCEPTIBILITY OF *ANOPHELES QUADRIMACULATUS* AND *A. ALBIMANUS* TO DOMESTIC AND FOREIGN STRAINS OF *PLASMODIUM VIVAX*

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During recent years many studies have been directed toward determining the potential ability of mosquitoes indigenous to the United States to transmit malaria of foreign origins. This interest has been dictated by the return to this country of exotic strains of malaria by military forces. In their studies on imported malarias, Young and his associates (1945, 1946, 1946, 1948) investigated thoroughly the infectivity of these foreign *vivax* malarias to domestic vectors.

During subsequent years investigations of this type have been continued, and have been extended to include studies of the comparative susceptibility of *Anopheles quadrimaculatus* and two strains of *A. albimanus* to one domestic and two foreign strains of *Plasmodium vivax*. One of the foreign *vivax* malarias, the Chesson, was originally from the South Pacific (New Guinea) area; the second originated in Korea and was derived from returning military personnel. The domestic (St. Elizabeth) strain has been in use for a number of years in these laboratories for treatment of neurosyphilis.

Young and Burgess (1952) reported the infectivity of Korean *P. vivax* to *A. quadrimaculatus*. Since this malaria is currently being introduced in this country by returning military personnel, further knowledge of its ability to infect various mosquitoes is important.

METHODS

The various strains of *P. vivax* were maintained in neurosyphilitics undergoing malaria therapy, inoculated either by infected blood or by the bites of infected mosquitoes.

Three strains of *Anopheles* were used in the study: *A. quadrimaculatus* (Q-1 strain) and two strains of *A. albimanus*, one from Panama (A-2 strain) and one from the Florida Keys (A-3 strain).

The procedures consisted of feeding simultaneously lots of about 100 specimens from each of two or three of the anopheline strains on patients infected with the *vivax* strains. The engorged specimens were maintained in an insectary at 74–78°F. and were dissected and the oocysts counted 10 ± 1 days after the blood meal. An average of 27.1 individuals per lot was dissected.

RESULTS

St. Elizabeth strain of P. vivax. Paired feedings compared the susceptibility of *A. quadrimaculatus* with the two strains of *A. albimanus*. Table 1 summarizes the results of these feedings.

In 24 feedings with *quadrimaculatus* (Q-1) and the Panama *albimanus* (A-2), the former averaged 70.9 per cent infected, with a mean oocyst density of 42.2

TABLE 1

Relative susceptibility to the St. Elizabeth strain of P. vivax *of* Anopheles quadrimaculatus *(Q-1) and the Panama (A-2) and Florida Keys (A-3) strains of* A. albimanus

No. of Feedings	Mosquito	No. Diss.	Per Cent Infected	Average Oocysts*	Gut Infection Index†
24	Q-1	678	70.9	42.2	2992.0
	A-2	557	0.07	6.0	0.4
15	Q-1	383	61.7	17.4	1073.6
	A-3	300	0	0	0.0

* Per infected gut.

† No. of oocysts in 100 total guts.

TABLE 2

Relative susceptibility to the Chesson strain of P. vivax *of* Anopheles quadrimaculatus *(Q-1) and the Panama (A-2) and Florida Keys (A-3) strains of* A. albimanus

No. of Feedings	Mosquito	No. Diss.	Per Cent Infected	Average Oocysts*	Gut Infection Index†
33	Q-1	794	83.5	208.3	17393.1
	A-2	1557	1.6	10.2	16.3
17	Q-1	508	85.0	246.6	20961.0
	A-3	585	1.8	5.2	9.4
17	A-2	586	0.8	3.0	2.4
	A-3	585	1.8	5.2	9.4

* Per infected gut.

† No. of oocysts in 100 total guts.

per infected gut. In only one feeding was a single specimen of Panama *albimanus* infected. The presence of six oocysts in this specimen resulted in a deceptively high mean oocyst number; the comparative susceptibility is more realistically shown by the gut infection index.

Fifteen feedings comparing *A. quadrimaculatus* with the Florida Keys *albimanus* (A-3) resulted in a mean infection rate of 61.7 per cent in the *quadrimaculatus*, with an oocyst rate of 17.4 per infected gut, while no *albimanus* infections were found.

Chesson strain of P. vivax. Paired feedings determined the relative susceptibility in all combinations of the three anophelines. Table 2 presents the results of the paired feedings.

In 33 feedings *A. quadrimaculatus* had a mean infection rate of 83.5 per cent and an average of 208.3 oocysts per infected gut; Panama *albimanus* averaged only 1.6 per cent infected and 10.2 oocysts per infected gut.

Similarly, *A. quadrimaculatus* was far superior to the Florida Keys *albimanus* in susceptibility. In 17 feedings the *quadrimaculatus* were 85.0 per cent infected with an average of 246.6 oocysts. The Florida Keys strain averaged only 1.8 per cent infected, and had a mean oocyst density of 5.2 per infected gut.

TABLE 3

Relative susceptibility to Korean P. vivax *of* Anopheles quadrimaculatus (*Q-1*) *and the Panama* (*A-2*) *and Florida Keys* (*A-3*) *strains of* A. albimanus

No. of Feedings	Mosquito	No. Dias.	Per Cent Infected	Average Oocysts*	Gut Infection Index†
30	Q-1	464	80.4	66.9	5378.8
	A-2	750	0	0	0.0
7	Q-1	146	60.6	15.7	951.4
	A-3	145	0	0	0.0

* Per infected gut.

† No. of oocysts in 100 total guts.

Comparison of the two strains of *A. albimanus* in 17 paired feedings yielded low rates of infection and mean oocysts densities in both strains. The Florida Keys *albimanus* apparently was slightly superior in susceptibility, with a mean rate of 1.8 per cent as compared to 0.8 per cent in the Panama strain. The oocyst densities followed the same pattern, with an average of 5.2 in the Florida Keys and 3.0 in the Panama strain.

Korean strains of P. vivax. Korean malarias from several sources were used in the relative susceptibility studies. Paired feedings compared the susceptibility of *A. quadrimaculatus* with that of the two strains of *albimanus*. Table 3 summarizes the results of these experiments.

It can be seen that in neither of the two *albimanus* strains were infections found, even though the *quadrimaculatus* were uniformly quite heavily infected. Transmission of the Korean strains was easily effected with *A. quadrimaculatus* in all cases tried.

DISCUSSION

Boyd and his coworkers (1938) reported very low rates of infection in a Panama strain of *A. albimanus* when fed on a Florida strain of *P. vivax* highly infective to a *A. quadrimaculatus*. Our results with another U. S. strain of *vivax* were similar, and extended these findings to a strain of *albimanus* originating in the Florida Keys.

Young *et al.*, (1946), using a strain of *A. albimanus* from the Brownsville, Texas, area reported very low rates of infection with the Chesson strain of *P. vivax*. In their experiments only 1.4 per cent of the *albimanus* became infected while 68.8 per cent of *A. quadrimaculatus* were infected in simultaneous feedings. Similar results were obtained in the present study using Panama and Florida Keys strains of *albimanus*.

The present report confirms that of Young and Burgess (1952) in which the infectivity of Korean *vivax* strains to *A. quadrimaculatus* was demonstrated. It is possible that the importation of Korean strains of *vivax* may pose a real problem in malaria transmission within the U. S. Unpublished studies in our laboratories indicate that there is little or no cross immunity between the Korean strains and the native U. S. (St. Elizabeth) or the Chesson strains of *vivax*. The Korean

vivax is similar to the St. Elizabeth strain in virulence and in relapse pattern. This relapse pattern, with an eight or nine month latent period, is particularly well suited to temperate climates similar to those found in areas of the United States where *A. quadrimaculatus* may also be found.

If other strains of *A. albimanus* are similar to those tested for susceptibility to the Korean *vivax*, it would be unlikely that this malaria is a potential hazard to areas where this anopheline is the principal vector. The studies of Boyd *et al.*, (1938) and Young *et al.*, (1946, 1948), in addition to the present report, indicate that *A. albimanus* is in general not receptive to nonindigenous strains of *P. vivax*. On the other hand, all of these same studies have indicated that *A. quadrimaculatus* is quite receptive to many strains of *P. vivax*, both domestic and foreign. In fact, to our knowledge, with no strain of *vivax* or *falciparum* malaria ever tested has *A. quadrimaculatus* been to any significant extent refractory to infection. In several cases (Boyd *et al.*, 1938, 1940) *quadrimaculatus* evidenced a higher degree of susceptibility to foreign strains of *P. falciparum* and *P. vivax* than did *A. albimanus* coindigenous with the malaria strains.

SUMMARY

The relative susceptibility of *Anopheles quadrimaculatus* and two strains of *A. albimanus* (Panama and Florida Keys strains) to three strains of *Plasmodium vivax* was determined. The strains of *vivax* included a U. S. strain (St. Elizabeth), a New Guinea strain (Chesson), and *vivax* of Korean origin.

In all cases *A. quadrimaculatus* showed a high degree of susceptibility, while the two strains of *albimanus* either were completely non-susceptible, as in the Korean *vivax*, or became infected only occasionally and at a very low rate.

The infectivity of Korean *vivax* to *A. quadrimaculatus*, and transmission through that species, confirms the possibility of establishment of malaria from that area in this country.

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MILK DIET AND HUMAN MALARIA

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The studies of Maegraith *et al.* (1952), MacKerras (1953), Ramakrishnan *et al.* (1953), Rodhain (1953) and Hawking (1954) indicate that a milk diet has a suppressive effect on malaria infections in rats, monkeys and mice. Further, that a milk diet deficient in p-aminobenzoic acid and possibly other factors exerts this effect by depriving the malaria parasite of nutritional entities essential to its growth. There is no information on the influence of a milk diet in human malaria, nor is it known if a diet rich in p-aminobenzoic acid favors the multiplication *in vivo* of human species of malaria. In an attempt to investigate these problems, African children naturally infected with malaria were placed on alternating periods of milk diet and normal diet, and for part of the time during the normal diet period, yeast was added, a recognized rich source of p-aminobenzoic acid.

MATERIALS AND METHODS

Nine children ranging in age from four to seven years were chosen for the experiment. All harbored infections with *Plasmodium falciparum* or *P. malariae*, or with both species. When not receiving milk the children were fed a basic diet of partially polished rice, fresh or tinned fish, and palm oil. This was supplemented during specific periods with yeast, each child receiving daily six 0.4 gm. tablets (total 2.4 grams) of yeast. The yeast tablets (product of Mead, Johnson & Co.) were crushed, mixed with peanut butter and fed on a graham cracker.

Milk was fed for three-week periods; a commercial brand of reconstituted whole milk powder was used. The children were offered and usually took eight glasses or approximately 64 ounces daily. Granulated sugar was added to every second glass. It was estimated that each child received from 1,500 to 2,000 calories daily.

The children were divided into two groups on the basis of whether they were negative or positive for malaria at the start of the experiment. *Group A* included six children. They received a diet as follows: a normal diet for the first eight days followed by a period of 21 days when only milk and sugar were fed; this in turn was followed by a 21-day period of normal diet at the end of which the children were again fed only milk and sugar for the final 21 days of the experiment. During the normal diet period of 21 days they received a yeast supplement for a ten-day period. *Group B* included three children. They received the following diet: a normal diet for the first 50 days followed by a period of 21 days when only milk and sugar were fed. The normal diet was supplemented with yeast on two occasions for periods of 14 and 10 days respectively.

While the children were on the milk diet they received no other food than the granulated sugar. They were isolated in a bungalow on the Institute grounds and were under constant supervision day and night. It is most unlikely therefore

that any other food was taken. Further, it should be pointed out that during the course of the experiment there was very little possibility that the children were exposed to further malaria infection. The bungalow where they lived was completely and properly screened and they were never outdoors after dark; also mosquitoes are scarce around the bungalows on the Institute grounds.

The parasite density was determined daily for each child. Blood for a thick smear was taken from all children each day and the parasite/white-blood-cell ratio determined by direct counts; gametocytes of *P. falciparum* were not included in the counts, but no attempt was made to exclude *P. malariae* gametocytes. White-blood-cell counts were made every second day. The parasite density which is expressed as the number of parasites per cubic millimeter of blood, was determined by multiplying the parasite/w.b.c. ratio by the w.b.c. count.

The children were under the constant supervision of a nurse who took temperature readings at least twice daily at 7.00 A.M. and 6.00 P.M., and more often if a child complained of headache or fever. All complaints of ill health were carefully recorded. All children were weighed daily. The spleen size (measurements of Hackett) was determined for each child at weekly intervals. The experiment continued for 71 days.

RESULTS

Group A

Five of the six children in this group showed both *P. falciparum* and *P. malariae* in their blood, and the sixth, *P. malariae* parasites alone. In three of the five with the double infections *P. falciparum* predominated, while in the remaining two both species predominated at different times. The parasite and clinical histories of the six cases are detailed briefly below.

Case #1. Tucker: male, 5 yrs. (*Fig. 1*). *P. falciparum* parasites were seen on all blood smears from this child and predominated throughout. On several smears parasites of *P. malariae* were seen but always in low numbers. The parasite density fluctuated throughout the course of the experiment, but there was no apparent correlation with either the milk diet or yeast feeding. The spleen size ranged from 1 to 3 and was found to be 2 on the majority of examinations. The patient showed clinical malaria on two occasions, once during the initial eight-day period of normal diet and again during the final milk diet period.

Case #2. Marmadee: male, 6 yrs. This child showed *P. falciparum* paraparasites in the blood on all examinations and this species predominated throughout. *P. malariae* parasites appeared during the latter half of the experiment but never comprised more than about one-fourth of the total parasites present. There was no apparent correlation between parasite numbers seen in the blood and the type of diet. The spleen size which was 2 on initial examination fluctuated between 0 and 2 throughout the course of the experiment but with no apparent relationship to the type of diet. There were two clinical attacks of malaria, both occurring during the course of the first milk-diet period.

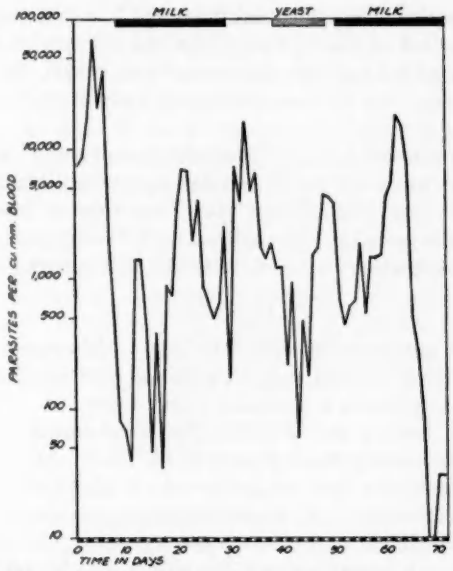

FIG. 1. Daily malaria parasite densities in case #1 (Tucker) receiving a regular diet, alternating with periods of milk and regular diet plus yeast.

Case #3. Enders: female, 6 yrs. P. falciparum parasites were seen on all blood smears taken from this child. During the final two weeks of the experiment parasites of *P. malariae* were also seen but in small numbers. The milk diet and the feeding of yeast had no apparent effect on the parasite density. The spleen size measured 3 at all examinations. There was one clinical attack of malaria which coincided with the beginning of the first milk-diet period.

Case #4. Jallah: male, 6 yrs. This child had a consistently high parasitemia and almost invariably showed parasites of both *P. falciparum* and *P. malariae* in his blood. *P. falciparum* predominated for roughly the first and last thirds of the experiment, while *P. malariae* parasites were more numerous during the middle third. There was no apparent correlation between the type of diet and the parasite density. During the first half of the experiment the spleen could not be palpated, but for the last half it fluctuated in size between 2 and 3. Clinical attacks of malaria occurred on three ocasions, once during the second milk-diet period and the other two times during the normal-diet period.

Case #5. Viola: female, 6 yrs. For the first six days this child showed only a very few gametocytes of *P. falciparum*. On the seventh day *P. malariae* parasites appeared in the blood and were seen on all subsequent examinations. About midway in the experiment parasites of *P. falciparum* made their appearance and for a period of approximately two weeks predominated; following this they fell off in numbers and *P. malariae* again became the predominant species although present in small numbers. The type of diet had no apparent influence

on the parasite density. The spleen size measured 3 or 4 throughout the experiment. During a period of about three weeks which extended through much of the first milk-diet period and into the normal diet period, the patient suffered from clinical malaria. For the remainder of the experiment she was clinically well.

Case #6. Careysburg: male, 7 yrs. This child showed only *P. malariae* parasites in his blood. There was no apparent correlation between diet type and parasite density. The spleen size ranged from 1 to 3. There were no symptoms of clinical malaria, but on one occasion, during the first milk-diet period, the child was found to have a low-grade fever which coincided with a parasite density peak.

Group B

One child in this group was infected with both *P. falciparum* and *P. malariae*. The other two children showed only *P. malariae* parasites in their blood. The parasite and clinical histories are detailed briefly below.

Case #7. Fegbi: male, 4 yrs. (*Fig. 2*). The blood smears of this child were negative for parasites until the sixth day of the study. At that time parasites of *P. falciparum* appeared and predominated on all subsequent blood smear examinations. Parasites of *P. malariae* first appeared toward the end of the first half of the experiment and were always seen in the blood from that time to the end, but never in large numbers. The spleen, which could not be palpated

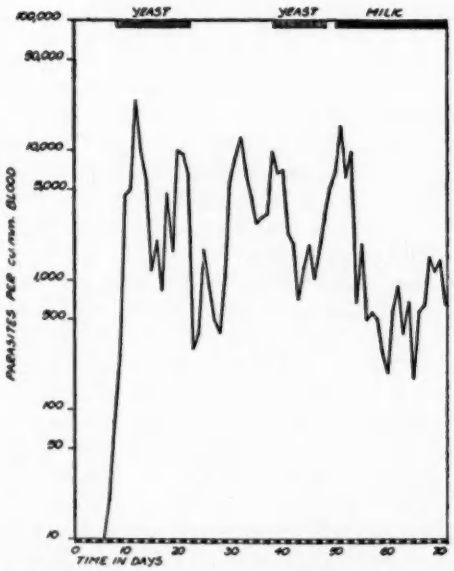

FIG. 2. Daily malaria parasite densities in case #7 (Fegbi) receiving a regular diet, alternating with periods of milk and regular diet plus yeast.

at the end of the first week, enlarged to a size 3 by the end of the second week and measured 2 or 3 on all subsequent examinations. There were two clinical attacks of malaria, one during the normal-diet period and the other during the early part of the milk-diet period.

Case #8. Armah: male, 5 yrs. P. malariae parasites only were seen in the blood of this child. They first appeared on the ninth day and were present on all subsequent blood examinations. The spleen, which was negative for the first two weeks enlarged to size 4 by the end of the third week and measured 3 or 4 on all further examinations. Two episodes of clinical malaria occurred during the normal diet-period.

Case #9. Ellen: female, 7 yrs. This child showed three brief episodes of parasitemia with *P. malariae*. They appeared in the milk-diet period as well as in the normal-diet period. The spleen was never palpable, and there were no clinical manifestations of malaria at any time.

DISCUSSION

The results of the present study demonstrate that within the conditions of the experiment a milk diet, or the addition to a normal diet of yeast which is rich in p-aminobenzoic acid, do not influence the parasite density or the clinical manifestations of *P. falciparum* and *P. malariae* infections in semi-immune children. And it would appear that a milk diet is of no practical importance in the treatment of infections with the above-mentioned species of malaria.

Admittedly, the results of this experiment are not strictly comparable with those obtained by investigators working with rat and monkey malaria. Nevertheless, our results do suggest that the nutritional deficiencies induced in the host by a diet essentially of milk alone, do not affect the multiplication of *P. falciparum* and *P. malariae* in the human host, as *P. berghei*, and *P. knowlesi* and *P. cynomolgi*, apparently are affected in rats and monkeys respectively. It is possible that the powdered milk used in our experiment in the amounts taken may have contained adequate quantities of p-aminobenzoic acid and other essential substances. On the other hand one cannot overlook the possibility that different species of malaria may have somewhat differing nutritional requirements, or that certain host species are more efficient in supplying vital nutrients to their malaria parasites in the face of a deficient diet.

SUMMARY

The effects of a whole-milk diet, and of a normal diet supplemented with yeast known to be a rich source of p-aminobenzoic acid, were tested in nine African children infected with *P. falciparum* or *P. malariae* or with both species. The results demonstrate that within the conditions of the experiment neither a milk diet, nor the addition of yeast to a normal diet, influence the malaria parasite density or the clinical manifestations of human malaria. These findings are at variance with those reported by investigators working with malaria species infecting rats and monkeys.

ACKNOWLEDGMENTS

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THE SUPPRESSIVE TREATMENT OF NATURALLY ACQUIRED MALARIA IN A RURAL VILLAGE WITH PYRIMETHAMINE (DARAPRIM)

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The village used in this experiment was Nuevo Vigia. It is a comparatively new village on the bank of Madden Lake, about 3 miles above the old village known as Vigia. A small stream, Quebrada Melgada, empties into Madden Lake after passing through the town. Before Madden Lake was formed it was a branch of the Pequeni that entered the Rio Chagres not very far above the site of Nuevo Vigia. The Trans-isthmian highway passed within two miles of the town and by automobile it is an hour's ride from Panama City to the village. The people who live around the Madden Lake used to cross the lake in their boats and unload at the Madden Dam where by bus transportation they sent their produce to the markets in either Panama or Colon.

Recently this area was made a military reservation, so the lake shore residents now use pack horses on trails to the nearest contact with the highway. A number of small villages are located on the lake above Nuevo Vigia so that many rural people pass through the place on the way to the Trans-isthmian highway. No form of antimalarial control was in use so that a favorable opportunity was provided to attempt suppression of malaria infection with pyrimethamine.

Suppressive treatment was started on Jan. 20, 1954 and ended May 19, 1954, a period of 18 weeks. Weekly doses of 25 milligrams were given to all adults and to all children above 2 years of age. Half this dose was given to children under 2 years of age. Our field book shows the names of 376 people, but only 134 of them were regular in their attendance on treatment days.

Two initial blood film surveys revealed the following infection:

	No. Examined	No. Positive for Malaria	Parasite Species			Per Cent Positive
			F.	V.	Mixed	
Adults	49	12	12	0	2	24.3
Children under 12 years	85	35	11	21	1	41.2
Total	134	47	23	21	3	35.1

As a rule, after three regular weekly treatments, the weekly thick blood film surveys were negative for parasites, but in twelve cases the gametocytes of *P. falciparum* persisted for some time: in 5 individuals for three consecutive weeks; in 3 for four weeks; in 2 for five weeks; and in 2 for six weeks.

Most of these individuals were children under 2 years of age, yet they took their treatment regularly. No difficulty was encountered in administration of the drug, for it is almost as tasteless as chalk and we had no toxic effects to

record. There were two people among the irregular visitors who had *P. vivax*. Each had had three successive weekly treatments and the third weekly survey recorded a few gametocytes.

SUMMARY AND CONCLUSIONS

Pyrimethamine has no unpleasant taste. Some babies were given crushed tablets with a drink of water to wash them down. No toxic effect was noted. Of the 47 who received weekly doses regularly, twelve revealed the gametocytes of *P. falciparum* in their blood films for two to six weeks. Blood films on all of the 134 regular people were negative at the end of the experiment. No clinical symptoms or relapse occurred during the 18 weeks of suppressive treatment in any of the treated people after the first 3 weeks, when all became free from asexual forms. This appears to be a very good drug for suppressive use if continued weekly. We get the same result with chloroquine but pyrimethamine is easier to administer.

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THE TREATMENT OF ACUTE MALARIA WITH SINGLE ORAL DOSES OF AMODIAQUIN, CHLOROQUINE, HYDROXYCHLOROQUINE AND PYRIMETHAMINE[1]

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During the past few years, a comparison has been made in the Company's hospital in Honduras of the efficacy of several antimalarial drugs when given in a single oral dose to patients with acute *Plasmodium falciparum* or acute *Plasmodium vivax* malaria. At its beginning, the study included only amodiaquin (Camoquin®) and chloroquine (Aralen®); an earlier publication (Hoekenga, 1952) reported 600 of these cases. Later, hydroxychloroquine (Plaquenil®) was added (Hoekenga, 1953). Still more recently pyrimethamine (Daraprim®) has been employed. In all, this paper concerns 1,020 malaria infections treated by the single dose method with one or other of these four compounds.

PHARMACOLOGIC AND CLINICAL PROPERTIES OF THE DRUGS

The three 4-aminoquinolines (amodiaquin, chloroquine and hydroxychloroquine) have the following properties in common: (1) they are rapidly and completely absorbed from the gastrointestinal tract of experimental animals and presumably of man; (2) they are distributed unequally in the body and are present in much higher concentrations in some tissues than in blood plasma; (3) they are slowly excreted from the body on cessation of therapy; (4) they are rapidly effective in terminating acute attacks of malaria caused by *P. falciparum*, *P. malariae*, or *P. vivax*; (5) they will eradicate *P. falciparum* infections when used in adequate dosage; (6) they decrease the number and frequency of *P. vivax* relapses; (7) as a group they do not have any recognizable gametocidal effect in *P. falciparum* infections; (8) they have little or no effect against sporozoites; and (9) they rarely cause toxic effects in therapeutic dosage.

Chloroquine, 7-chloro-4-(4-diethylamine-1-methylbutylamine) quinoline diphosphate, emerged as one of the more active and less toxic quinoline derivatives in the World War II evaluation of potential antimalarials. Consequently it has been used widely with satisfactory results in thousands of infections. It is supplied in 0.25 gm. tablets as the diphosphate, representing 0.15 gm. (or 60%) chloroquine base. Recommended treatment of malaria consists of administration of 4 tablets as an initial dose, followed within 6 to 8 hours by 2 tablets and 2 tablets on each of two succeeding days.

Amodiaquin, 7-chloro-4-(3-diethylaminomethyl-4-hydroxyaniline)quinoline dihydrochloride, synthesized in 1943, is low in toxicity and has been used in many

[1] Portions of this paper were included in addresses presented at the Fifth International Congresses of Tropical Medicine and Hygiene, Istanbul, August 30, 1953; at the annual meeting of the American Society of Tropical Medicine and Hygiene, Louisville, November 12, 1953; and at the Inter-American Medical Convention, Panama City, March 25, 1954.

parts of the world with good results in dosages even smaller than those recommended for chloroquine. It is supplied as the dihydrochloride dihydrate in tablets of two sizes: 0.15 gm. and 0.2 gm. of the base. Recommended treatment is 0.6 gm. as a single dose.

Hydroxychloroquine, 7-chloro-4-[4-(N-ethyl-N-β-hydroxyethylamino)-1-methylbutylamino] quinoline diphosphate, was synthesized in 1950. Clinical trials have shown that in a single dose of 1.25 gm. it is as efficient as chloroquine and amodiaquin, and without any reported toxic effects. The minimal effective dose has not yet been determined.

Pyrimethamine, 2:4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine, is the most active of an entirely new class of antimalarials. Some 300 of these compounds were synthesized in 1949–1950 as an outgrowth of studies by Hitchings, Falco and co-workers (1945, 1949, 1950, 1951, 1952) on antagonists to nucleic acid. The search for such antagonists was founded on the thesis that there must exist a differential requirement between host and parasite for the nucleic acid precursors involved in growth. Early studies showed a high potency of pyrimethamine against laboratory malarial infection, being 60 times as active as chlorguanide against *P. gallinaceum* in chicks and 200 times as active against *P. berghei* in mice. Clinically it has proved to be an excellent suppressant-prophylactic, even in a very small oral dose of 25 mg. at weekly intervals. It is claimed to be effective in controlling acute malarial attacks in a pre-immunized native population; it slowly sterilizes the tissue forms of *P. vivax*, producing "suppressive cures" when the recommended suppressive regimen is continued for eight weeks or longer (Coatney *et al.*, 1953); and it renders *P. falciparum* gametocytes lastingly sterile (Foy *et al.*, 1952). Pyrimethamine is tasteless and odorless. It is supplied in tablets of 25 mg. The recommended treatment for a clinical attack of malaria is one or two 50 mg. doses. With these quantities, no toxic effects have been noted.

MATERIAL AND METHODS

This study reports the treatment of 1,020 patients hospitalized with acute malaria. All of them resided in or near the Tela Railroad Company (United Fruit Company) headquarters at La Lima in northwestern Honduras. In this area *Anopheles albimanus* is the principal vector and malaria is endemic all year, with slightly decreased incidence during the dry season from May to September. Because malaria is endemic, the adult native population may be considered to have acquired partial immunity. Eighty per cent of patients treated were males and twenty per cent females. The ages ranged from 7 to 65 years. All were of mestizo stock and represented various mixtures of Indian, Negro and Spanish ancestry.

Chloroquine was administered to 320 patients in 2 single dose schedules: 0.45 gm. of base or 0.6 gm. of base.

Amodiaquin was given to 500 patients divided in four groups according to single dosage used. The individual members of each group received 0.4 gm., 0.6 gm., 0.8 gm. or 1.0 gm., respectively, of the base.

The 125 hydroxychloroquine-treated patients received 1.25 gm. in a single oral dose.

Eighteen persons received a 50 mg. dose of pyrimethamine and 57 received a 100 mg. dose.

As soon as the diagnosis was confirmed by blood film, treatment was begun. Oral temperature on all patients was recorded every four hours. Throughout hospitalization, thick blood films were examined twice daily in chloroquine and amodiaquin groups, four times daily in the hydroxychloroquine and the pyrimethamine-treated patients. The amount of drug and the time of administration were not related to parasite density, to the stage of parasite cycle, or to the degree of fever. All groups were comparable in respect to degree of parasitemia when treatment was started.

RESULTS

Clinical response. In nearly all patients treated with one of the 4-aminoquinolines, headache, malaise, vomiting, joint pains and other symptoms caused by malaria disappeared within 36 to 48 hours and patients were usually able to return to work by the fourth day after treatment. The response to pyrimethamine was less rapid and convalescence was prolonged by one to three days. Temperature returned to normal earlier in patients with *P. falciparum* malaria receiving 1.0 gm. of amodiaquin base than in patients given lesser amounts of this drug; this was not noted in patients with *P. vivax* malaria. Table 1 shows that the

TABLE 1

Results of 1,020 single dose treatments of malaria*

Doses Gm. of Base	Total Patients	*P. falciparum* Infection			*P. vivax* Infection		
		Number of patients	Average number of hours until		Number of patients	Average number of hours until	
			Temp. remained normal	Parasites disappeared		Temp. remained normal	Parasites disappeared
Amodiaquin	500	243	—	—	257	—	—
1.0	75	33	13.2	26.3	42	15.3	27.5
0.8	80	40	18.5	34.6	40	14.6	25.6
0.6	245	120	17.8	31.0	125	17.2	25.3
0.4	100	50	19.8	30.7	50 (3)†	18.2	27.6
Chloroquine	320	150	—	—	170	—	—
0.6	150	70 (1)†	19.1	30.0	80	16.5	23.4
0.45	170	80 (5)†	32.1	38.2	90 (4)†	20.4	29.8
Hydroxychloroquine	125	50 (2)†	20.9	30.3	75	18.7	24.0
Pyrimethamine	75	34	—	—	41	—	—
0.1	57	32 (2)†	36.5	38.4	25 (1)†	31.9	34.2
0.05	18	2	—	34.0	16 (3)†	30	34.5

* Late relapses are not indicated in this table.

† The figure in parenthesis represents the number of patients in which the response to the single dose treatment was not satisfactory. These immediate failures to the single dose treatment are not included in the tabulation of the average number of hours until temperature remained normal nor the average number of hours for parasite clearance.

0.45 gm. dose of chloroquine did not prove entirely satisfactory as far as temperature response is concerned, and that the recommended amounts of pyrimethamine gave results generally inferior to the 4-aminoquinolines.

Parasitological response. Table 1 includes the number of hours required for disappearance of parasitemia on each of the dosage regimens. The persistence of *P. falciparum* gametocytes is not included in the tabulation; precise records on gametocyte clearance were not kept. Gametocytes of *P. vivax* usually disappeared promptly, but *P. falciparum* gametocytes often persisted until subsequent treatment with primaquine or pentaquine. Among the 34 *P. falciparum* cases treated with pyrimethamine, gametocytes appeared after treatment in 6 instances and stubbornly persisted; it was not possible to do experiments to show whether or not these gametocytes had been rendered lastingly sterile—one of the properties which the drug is alleged to possess.

Treatment failures. Among the 320 patients who received chloroquine, 10 did not respond (one from the 0.6 gm. group, and nine from the 0.45 gm. group), Table 1. These ten continued to have schizonts in the peripheral blood as well as temperature elevation and other clinical symptoms that necessitated retreatment. All responded satisfactorily to the recommended 3-day chloroquine regimen.

In the amodiaquin groups of 500 patients there were only three who did not respond, all of whom had been given the 0.4 gm. dosage. They reacted promptly to treatment with 1.0 gm. of amodiaquin.

In 125 patients on the hydroxychloroquine regimen, two immediate failures occurred as shown in Table 1.

Among the 75 pyrimethamine-treated individuals, there were six immediate failures.

Relapses. The number of known relapses is small. Only 16 persons reported to the hospital or outpatient dispensaries with return of symptoms and parasitemia. All had malaria due to *P. vivax*. Seven had been treated with 0.45 gm. of chloroquine, three with 0.6 gm. of chloroquine, and six with 0.4 gm. of amodiaquin. There were no relapses reported among the 125 hydroxychloroquine-treated patients nor among the 75 pyrimethamine-treated ones.

Toxicity. In the dosages administered, no serious toxic effects were noted. Five chloroquine-treated patients and five of those who received amodiaquin vomited after receiving the drug, but all ten of them tolerated readministration of the same medication and so it is more logical to ascribe the vomiting to the disease than to the antimalarial drugs. All four compounds used in this study were given to persons with malaria complicated by other conditions such as cardiovascular disease, marked malnutrition or cachexia, hepatic or renal disease, pulmonary diseases, or advanced pregnancy. In no instance was there clinical evidence of drug toxicity or intolerance.

COMMENTS

It should be emphasized that this study was done in an area where malaria is endemic and the adult indigenous population presumably has acquired a well-

developed partial immunity. Owing to this fact, results of treatment in a non-immune population or in an immune population of another location might be different. Variation in parasite strain might also produce different responses.

As a result of this study in this locality it now appears practical to treat most patients with malaria on an ambulant basis with a single dose by mouth of a few tablets of either amodiaquin, chloroquine or hydroxychloroquine. This is also proving to be true in other endemic malaria areas, and is saving money, time and hospitalization. However, we do not advocate ambulant treatment for all cases; patients with *P. falciparum*, particularly, should be hospitalized for 36 to 48 hours when possible in anticipation of pernicious complications. Pyrimethamine can also be used for single dose treatment, but results in Honduras with recommended quantities are not equal to those obtained with the 4-aminoquinolines.

The time of disappearance of parasitemia is conservative in all cases, and especially in the chloroquine and amodiaquin groups; with blood films in these groups made only twice daily, the disappearance of parasites undoubtedly is seldom noted immediately after its actual occurrence. More frequently, a lapse of a few hours may be presumed to occur between the actual disappearance of parasites and its demonstration at the next blood examination.

No patients in this report received parenteral medication. Amodiaquin has been used parenterally with success elsewhere. Chloroquine has been injected intravenously and hydroxychloroquine both intravenously and intramuscularly, with success, here in Honduras. These later results will be transmitted in a separate communication. Reliable parenteral preparations such as these should be available for treatment of severely ill patients who, because of coma, vomiting or other complications such as sprue, are unable to take the medication orally.

In this report, an evaluation of the incidence of radical cures effected by these antimalarials has not been attempted. Such an evaluation is never satisfactory in an endemic malaria area because immunity cannot be measured nor reinfection closely controlled.

SUMMARY

One thousand and twenty Hondurans were treated for acute malaria with a single oral dosage of either amodiaquin (Camoquin), chloroquine (Aralen), hydroxychloroquine (Plaquenil) or pyrimethamine (Daraprim). In general, clinical response was excellent, treatment failures were few, relapses were infrequent, drug toxicity was negligible. The results with pyrimethamine were not equal to the results obtained with the three 4-aminoquinolines. Obviously, it is of great advantage to have several antiplasmodial agents that can be used in single oral dosage for mass treatment.

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SYMBIOSIS IN CULTURES OF *ENDAMOEBA HISTOLYTICA* AND SINGLE SPECIES OF BACTERIA

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In a previous paper by Rees, Baernstein, Reardon and Phillips (1953) it was shown that greater amounts of carbon dioxide were produced from cultures of *Endamoeba histolytica* with selected single species of bacteria than from the bacteria grown by themselves. In cultures of the ameba with *Clostridium perfringens*, these differences were insignificant and this was attributed to the utilization of rice starch by the bacterium. Controls showed that *C. perfringens* produced four times as much CO_2 in cultures with rice flour as in cultures without the rice, whereas the other species of bacteria that were studied produced about the same amounts of CO_2 in both cases. The requirement for rice starch by *E. histolytica* in cultures with single species of bacteria is well known and liberation of starch grains from rice flour in cultures of the ameba, and not by the bacteria without amebas, was demonstrated. Therefore, since our determinations of turbidity indicated richer growth of the bacterium in the presence than in the absence of amebas, a hypothesis was proposed that, through the fragmentation of rice flour and subsequent hydrolysis of the starch, *E. histolytica* produced substrate for the bacterium with a consequent richer growth and greater production of CO_2. No evidence of production of the gas by amebas *per se* could be obtained. It was probable, however, that in addition to what appeared to be a proteolytic enzyme causing fragmentation of the rice, the amebas might also produce amylase with consequent production of reducing sugar from the starch. Our interest in this enzyme was heightened by a report of Hallman and DeLamater (1953) on a diffusible amylase from cultures of *E. histolytica* with species of bacteria that were inhibited by antibiotics.

In the present investigation some analyses of medium for glucose and lactate were made in Eldredge-tube cultures of amebas and symbionts in attempts to ascertain relationships between amounts of glucose disappearing from the overlay during periods of incubation, and of CO_2 and lactate produced. The complicating factors of such a problem were obvious from the start. The specificity of applicable methods for analyzing glucose is not absolute; interchange of glucose between overlay of the medium and the base has been demonstrated (von Brand *et al.*, 1946), and it is difficult to evaluate the amount of glucose and other reducing sugar derived from the starch. However, the importance of information that might be obtainable in further confirmation of the previous report (Rees *et al.*, 1953), indicating symbiosis between *E. histolytica* and bacteria, appeared to justify the proposed experiments in spite of the complications. Enrichment of the medium with derivatives of glucose such as lactate, pyruvate, and 3-phosphoglyceric acid was tried in order to ascertain their effects on interactions of amebas and bacteria. Our efforts were also directed toward the demonstration of

amylase, since through hydrolysis of starch to reducing sugar this enzyme may account for substrate utilizable by the bacteria.

MATERIALS AND METHODS

Preparation of medium in Eldredge-tubes, measurement of CO_2, of growth of amebas, and of bacteria, were conducted as in the previous investigations, (Rees *et al.*, 1953). Total reducing substances were determined on tungstic acid supernates by the method of Folin and Malmros (1929). In later experiments a more specific method for measuring reducing sugar was used (Kingsley and Reinhold, 1949). Reducing sugars were calculated as glucose. Lactic acid was determined on protein-free supernates by the method of Barker and Summerson (1941), after removing interfering substances with $CuSO_4$ and $Ca(OH)_2$. The derivatives of glucose that were added to some of the cultures, lithium lactate, sodium pyruvate, and 3-phosphoglyceric acid, were commercial preparations. The barium salt of the last compound was converted to a soluble sodium salt with a calculated amount of Na_2SO_4. Production of amylase was investigated in standard cultures, and the overlay thereof by incubation with toluene, but for quantitative determinations, rich harvests of amebas were used. These were obtained from cultures in 1,000 ml. Florence flasks, containing 400 ml. of egg base and enough overlay, approximately 700 ml., to fill them to the neck. The amebas were collected by slow centrifugation and the sediment, containing about 10×10^8 total amebas, was suspended in 40 ml. acetone at 0°C., stirred occasionally during one hour, then centrifuged, the acetone discarded, the residue dried *in vacuo*, and stored at 0°C. Extracts were prepared by suspending the acetone powder in 25 ml. of a solution buffered at a chosen pH, stirring magnetically for 15 minutes, centrifuging, and collecting the supernate solution. The solution was used immediately for determinations of amylase activity. The digestion mixtures were composed of measured amounts of the extract adjusted to a selected pH plus 2 ml. of two per cent soluble starch, and water, to a total volume of 7 ml. The mixture was incubated at 37°C., and aliquots of 1.0 ml. withdrawn at intervals of 30 minutes. The protein of the aliquot was precipitated and removed by centrifugation, and the cleared fluid in amounts of 0.5 ml. was analyzed for glucose. Standard solutions of glucose and blanks were run simultaneously. From the concentration of glucose found, the average increase in glucose per hour was calculated. One unit of amylase was defined as the amount of the enzyme giving an increase of 50 μg. glucose per hour. The specific activity was expressed as units of amylase per mg. of protein in the digest. The amount of protein was ascertained by comparison with a standard by the method of Weichselbaum (1946). The standard was a solution of dried egg white containing 0.2 gm. per 100 ml. Total nitrogen of this solution was determined by micro-Kjeldahl, the factor 6.25 being used to calculate the amount of protein. Harvests of bacteria without amebas were used as controls for the above determinations.

RESULTS

Cultures of E. histolytica-*organism* t. The data summarized in Table 1 were obtained from two series of experiments, each series requiring 50 Eldredge tubes

TABLE 1

Analyses of cultures of Endamoeba histolytica *with organism* t, *compared with those of organism* t. *Averages of 10 cultures*

Hours Incubation	Turbidity, log I_0/I	Carbon Dioxide, μM	Glucose, μM	Lactate, μM	CO_2/T	L/T*	CO_2/G†	CO_2/G‡ Corr.	Ameba × 10^3
Endamoeba histolytica-organism *t*									
0	0.250	—	15.8	8.7	—	—	—	—	20.6
24	0.389	35.1	7.0	10.3	90	4.1	4.0	4.4	115.4
48	0.336	83.6	6.7	11.6	249	8.6	9.2	10.8	158.1
72	0.376	111.2	8.3	16.1	295	20.0	14.8	29.2	149.5
96	0.431	151.1	10.1	17.3	350	20.0	26.5	108.0	120.2
Organism *t*									
0	0.167	—	13.8	6.4	—	—	—	—	—
24	0.324	25.0	7.6	8.3	77	5.9	4.8	5.8	—
48	0.272	57.2	7.7	8.6	210	8.1	9.4	11.4	—
72	0.318	61.1	6.7	9.9	192	11.0	8.6	11.3	—
96	0.314	71.1	8.0	10.6	226	13.4	12.2	19.2	—

* Ratio of lactate produced to turbidity.

† Ratio of carbon dioxide produced to glucose disappearing.

‡ Same but corrected for lactate produced.

of medium. In each series 25 tubes were seeded with *E. histolytica*-organism *t*, and 25 with the bacterium alone. A control series of 25 tubes was kept sterile and analyzed for diffusion of glucose and lactate during storage for 96 hours. These results were utilized as a base line for calculations of the data of the above and other experiments. However, the usefulness of the data of the sterile series was limited by alterations in concentrations of glucose and lactate in the cultures of the respective organisms. The data of Table 1 were obtained from analysis of 5 sets of cultures at indicated periods of incubation. The data on turbidity, CO_2, and the ratios CO_2/T show general agreement with those of Rees *et al.* (1953) and calculations in micromoles used in present studies facilitates comparisons with glucose and lactate. The data on consumption of glucose by organism *t* show that the period of greatest activity occurred during the first 24 hours of incubation, and it is interesting that the growth of amebas occurred during the same interval. The growth of amebas increased rapidly during the first 24 hours and reached a seven-fold increase over the inoculum at 48 hours. Approximately half of the total reducing substances in the overlay disappeared during 24 hours. With the exception of the slight rise in the cultures of ameba at 96 hours, the residuum of reducing substance after 24 hours was probably composed of reducers other than glucose. The rise in the cultures with amebas may indicate a slow accumulation of reducing sugar due to increasing activity of amylase from the amebas.

The data show clearly that amounts of glucose disappearing from the overlay could not possibly account for all of the CO_2 produced, since the maximum ob-

TABLE 2

Analysis of cultures of Endamoeba histolytica-Aerobacter aerogenes *and of* A. aerogenes *at 96 hours of incubation at 37°C.*

Number of Experiments	Enrichments		Turbidity, log I_0/I	Carbon Dioxide Produced, μM	Glucose Used, μM	Lactate Produced, μM	CO_2/T	L/T	CO_2/G	CO_2/G Corrected for Lactate	Amebas × 10^3
	Compound	μM									
	E. histolytica-A. aerogenes										
4	None	—	0.519	200	8.0*	1.7	386	3.3	25	28	54.3
3	Glucose	26.4	0.493	227	19.3	2.1	460	4.2	12	12	51.0
2	Glucose†	26.4	0.471	142	17.4	10.3	302	21.9	8	11	35.5
6	Lactate	55.3	0.511	180	5.3	−36.5‡	352	−71.4‡	34	8	70.7
3	Pyruvate	53.9	0.455	219	8.3	−0.1	481	−0.2	26	8	69.0
2	3-phosphoglyceric acid	53.9	0.521	192	9.5	5.5	369	10.5	20	6	43.5
	A. aerogenes										
4	None	—	0.373	83	8.5*	−0.3	223	−0.8‡	10	10	
3	Glucose	26.4	0.421	133	20.0	1.7	315	4.0	7	7	
2	Glucose†	26.4	0.358	110	19.8	6.7	286	17.4	6	7	
6	Lactate	55.3	0.421	114	6.6	−37.1	270	−88.0	17	5	
3	Pyruvate	53.9	0.383	160	8.6	−0.2	418	−0.5	19	6	
2	3-phosphoglyceric acid	53.9	0.461	105	10.6	−1.4	227	−3.0	10	3	

* Calculated on the basis of amount in sterile medium plus amount in the inoculum.
† Incubated in an atmosphere of hydrogen.
‡ Indicates a decrease.

tainable under any conditions would not exceed a molar CO_2/G ratio of 6. Except at incubation for 24 hours when much of the CO_2 had not been absorbed in the $Ba(OH)_2$ of the Eldredge-tube, the ratios are higher than 6, particularly in the cultures with amebas, where at 96 hours the ratio was 26.5. It is highly improbable that this excess CO_2 was derived from protein or fat, although this possibility can not be excluded. When the ratios were corrected for lactate (column 9) the discrepancy between glucose disappearing and CO_2 produced was still higher. It is obvious that some sources of substrate can not be measured by the methods employed.

Cultures of E. histolytica-aerobacter aerogenes. The data on cultures of *E. histolytica-A. aerogenes* were obtained from 6 series of experiments, each series requiring 20 Eldredge tubes. Ten were inoculated with ameba-bacteria, and 10 with bacteria only. Five tubes of each set were analyzed within 30 minutes after seeding and 5 after incubation for 96 hours. The numbers of times that each experiment was repeated are shown in Table 2 which gives the averages of the determinations made.

The data on production of CO_2 in relationship to turbidity show essential agreement with those of Rees *et al.* (1953) in cultures without enrichment with glucose. The amount of glucose added in the present studies was not sufficient to obliterate the increases attributable to the ameba. Larger amounts, as was previously shown, would undoubtedly have done so.

Incubation in hydrogen instead of air showed some interesting differences. It must be emphasized that incubation in air under these conditions is not equivalent to aerobic cultivation since the amount of air was limited to that confined above the overlay. Even so, the removal of this small amount of air was sufficient to affect the amounts of CO_2 and lactate produced from approximately the same amounts of glucose used. It would appear that the loss of an aerobic path for reoxidation of diphosphopyridine nucleotide was compensated by an anaerobic conversion of pyruvate to lactate. Further, work is needed to establish this hypothesis.

Enrichment with lactate, pyruvate, and 3-phosphoglyceric acid showed essentially the same picture as enrichment with glucose. Calculations for pyruvate and 3-phosphoglyceric acid utilization were based on the assumption that all the material added was used. This was supported by pyruvate analyses but no determinations of 3-phosphoglyceric acid were made. The data are not sufficient to make profitable the discussion of the small differences observed.

The ratios CO_2/G, except in two cases, are equal to or greater than 6, indicating that there are sources of substrate that can not be measured by the methods employed. The exceptions noted above may be due to experimental error, but a ratio of 6 indicates the maximum value theoretically possible, and according to Umbreit (1952) the ratio for *A. aerogenes* is approximately 1.78. All ratios are far above this figure. The sources of substrate that could not be measured appear to reside in the egg base and the rice flour. The differences shown in Table 2 among the numbers of amebas growing in the cultures may or may not be significant since the amebas were counted at 96 hours of incubation, whereas the peak is known to occur between 48 and 72 hours. The principal significance of the figures arises from their demonstration that amebas grew in every case. In their entirety the data of Table 2 support the hypothesis of Rees *et al.* (1953) that *E. histolytica* furnishes substrate for growth of a commensal bacterium; in other words that there is symbiosis between *E. histolytica* and bacteria.

Experiments on amylase. In agreement with the above mentioned report of Hallman and DeLamater (1953), the data reported here indicate the occurrence of amylase in cultures of *E. histolytica* with species of bacteria, our work being restricted to cultures with organism *t*. In addition, as has already been indicated, we have investigated amylase from the ameba by methods designed to give quantitative data. The results of our investigations on amylase in the overlay of cultures are summarized in Table 3.

Table 3 shows that the overlay of the cultures of the ameba removed at 48 hours of incubation contained relatively little glucose compared with the amount present in sterile medium. During incubation, with soluble starch as substrate, the glucose content of this overlay increased progressively with the passage of time. No comparable increases occurred in the overlay from cultures of organism

TABLE 3

Demonstration of amylase by Endamoeba histolytica *from overlay of cultures with organism* t *removed at 48 hours of incubation. The substrate was one per cent soluble starch, and growth of organisms during incubation of the overlay was inhibited by toluene*

Hours of Incubation of the Overlay	Source of Overlay		
	E.h.-org. *t* glucose μg/ml	Org. *t* glucose μg/ml	Sterile medium glucose μg/ml
0	86	71	245
1	95	61	239
2	119	67	—
14	252	82	252

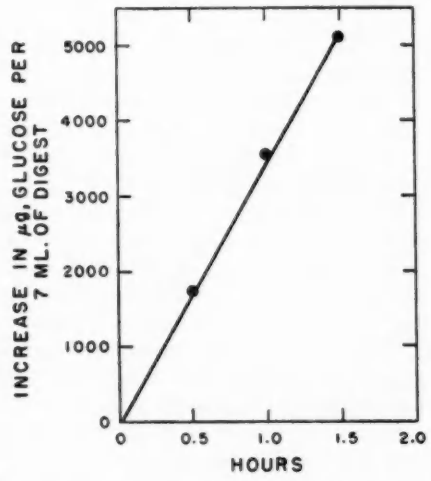

FIG. 1. Linearity of increase in glucose from extracts of *Endamoeba histolytica*. Digestion mixture prepared in 0.04 M phosphate. The components were 2.0 ml. extract, at pH 4.7; 2 ml. two per cent soluble starch; 3 ml. H_2O.

t indicating that no detectable amylase was produced by this bacterium. The increases in question could not be demonstrated in the absence of an inhibitor of glucose utilization.

The over-all methods of extracting the enzyme from harvests of amebas, of preparing the substrate, and of calculating the results of experiments have already been described. The results of our quantitative determinations of amylase are illustrated in Figures 1, 2, and 3. Linearity in effects of enzymatic hydrolysis of starch with the passage of time is illustrated in Figure 1. These data were obtained from 2.0 ml. of extract of acetone powder in 0.15 M phosphate buffer at pH 4.7. The figure shows that the increase in glucose was linear for at least 1.5 hours. Linearity was also observed over a range of pH from 3.32 to 7.20.

Figure 2 shows that the activity of extracts containing the enzyme was pro-

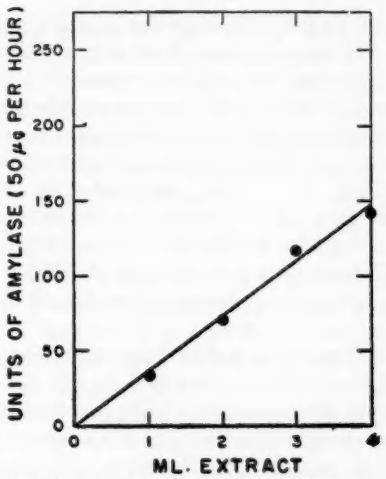

FIG. 2. Proportionality between activity of amylase and volume of extract of *Endamoeba histolytica*. The digestion mixture contained 2 ml., two per cent soluble starch, 1, 2, 3, and 4 ml. respectively of extract at pH 4.77; with sufficient H_2O for 7.0 ml. total volume.

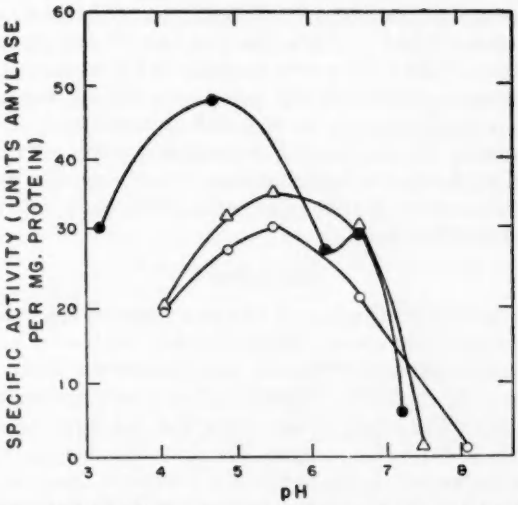

FIG. 3. Specific activity of digests from extracts of *Endamoeba histolytica* in relation to pH. ● Extract prepared in 0.042 phosphate; ○ in 0.028 M acetate; △ in 0.028 M acetate plus 0.01 M NaCl.

portional to the amount of extract used. This proportionality held up to a concentration of 2.8 mg. protein per 7 ml. of the digest.

For the data illustrated in Figure 3 on the relationship of pH to specific activity (units per mg. of protein) two extracts of acetone powder were prepared

as follows: (a) In 24 ml. of 0.15 M phosphate at pH 3.8, (b) in 25 ml. of 0.10 M acetate at pH 8.3. The extracts were stirred magnetically for 15 minutes and then centrifuged. The digestion mixtures were prepared as follows: (1) two ml. of 2 per cent soluble starch in water, with the pH adjusted by 0.1 N NaOH, and sufficient water added to make 5.0 ml. The reaction was started by adding 2.0 ml. of extract a; (2) two ml. of 2 per cent soluble starch in water with the pH adjusted with 0.1 N H_2SO_4. The reaction was started by adding 2 ml. of extract b; (3) two ml. of 2 per cent soluble starch in 0.038 M NaCl, with the pH adjusted by 0.1 N H_2SO_4, water to make 5 ml. The reaction was started by adding 2.0 ml of extract b. All mixtures were incubated at 37°C. and sampled every 30 minutes for 1.5 hours. Glucose was determined on 0.5 ml. of supernates from Cd SO_4, plus NaOH.

The results illustrated in Figure 3 show that with phosphate buffer the optimum pH was about 4.7 with a secondary peak at 6.7. Although this peak was observed later in another experiment with phosphate buffer, we can not be sure whether or not it is an artifact. The supernatants were analyzed by the method of Nelson (1944) and gave essentially the same picture as in Figure 3. With acetate buffer the optimum seemed to be shifted slightly toward higher pH, but further work is indicated to establish this shift with certainty. The absence of a secondary peak in incubates with acetate buffer also needs confirmation. There was about 15 per cent activation by 0.1 M NaCl on undialyzed extracts. Perhaps a greater activation would have been observed had the extracts been dialyzed. Although Myrback (1926) noted a shift in optimum pH on dialyzed salivary and pancreatic amylase, no such shift was noted by us in our material. Our best extract showed a specific activity of 48.4 units per milligram of protein. The The question whether the amylase of *E. histolytica* is extra- or intra-cellular can not be answered on the basis of available data. However, in investigations on the amylase of *Amoeba proteus*, Holter and Doyle (1938) found that the enzyme is associated with mitochondria.

DISCUSSION

A number of the complicating factors affecting appraisal and evaluation of the data herein presented have already been discussed. Analysis of reactions under these complications is far more difficult in cultures with the ameba than in those of a given symbiont by itself. This difficulty is particularly apparent in the analysis of the data of Table 2 where lactate, pyruvate, and 3-phosphoglyceric acid, respectively, were added to the medium. The data that were obtained on these substrates serve further to emphasize the need for pure cultures of *E. histolytica* for studies on its metabolism in relation to the metabolism of bacteria. However, since such cultures of the ameba are at present unobtainable, and since information on the metabolism of *E. histolytica* is urgently needed, we have attempted, with the use of a practicable method of measuring the output of CO_2 in Eldredge-tubes, and spectrophotometric and other chemical methods of measuring substrates and metabolites, to ascertain some of the interactions between the ameba and selected single species of bacterial symbionts. The experiments herein reported

on amylase produced by the amebas were beset with fewer complications than those on substrates and metabolites of growing organisms. However, such work is limited to studies on enzymes that may act independently of living cells. In addition to applications that may be made of the data thus far obtained in problems of amebiasis, our results may be of value as leads toward other methods of obtaining information on the metabolism of *E. histolytica* even with some of the standardized cultures now available.

SUMMARY

In cultures of *E. histolytica*-organism *t* and *E. histolytica-A. aerogenes* the production of carbon dioxide, measured in Eldredge-tubes, was greater than could be calculated on the basis of glucose disappearing and lactate produced. The results appear to indicate that some glucose which could not be measured was derived by diffusion from the solid phase of the medium, and from fragmentation of rice flour with hydrolysis of the starch thus liberated. The possibility that some of the carbon dioxide may have been derived from protein and/or fat could not be ruled out. The excess of carbon dioxide in cultures with amebas over that produced by the respective bacterial symbionts without amebas was attributed to production of sugar by the amebas and utilization thereof by the bacterium but some of the gas may have been produced by the amebas.

Lactate, pyruvate, and 3-phosphoglyceric acid provided substrates for growth of *A. aerogenes* in pure culture, but in cultures of *E. histolytica-A. aerogenes* there were factors that could not be analyzed.

E. histolytica was shown to produce amylase which finds its way into the medium by unknown pathways. Amylase was demonstrated in overlay removed from cultures of *E. histolytica*-organism *t* when growth of organisms was inhibited by toluene. Quantitative data on activity of amylase were obtained from extracts of precipitates by acetone of ameba-harvests. The best extracts showed a specific activity of 48.4 units of amylase per milligram of protein. The activity was highest in the presence of sodium chloride, and optimum activity occurred somewhere between pH 5 to 6.

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TRIAL OF DIALLYL-DIETHYL-AMINOETHYL PHENOL DIHYDROCHLORIDE (CAMOFORM) IN HUMAN AMEBIASIS[1, 2]

MARK T. HOEKENGA AND DOROTHY L. BATTERTON

The antiamebic compound, Camoform® (Parke, Davis & Co.) is a synthetic organic chemical possessing marked action against amebae pathogenic to rats, dogs, hamsters and man. In vitro it has an amebicidal power in dilutions as high as 1:50,000. It is a basic bisphenol that contains neither iodine nor arsenic in its molecule, the formula (diallyl-diethyl-aminoethyl phenol dihydrochloride) being structurally related to that of the antimalarial drug amodiaquin. Synthesized by Burckhalter and his co-workers (1946) this chemical is a white, crystalline substance readily soluble in water in excess of 10 per cent concentration. It has also been referred to as SN 6771 in the World War II malaria drug screening program, and by the Parke, Davis & Co. designation of PAA-701.

The following data are available (Parke, Davis & Co., 1953) on physiological distribution in man: during serial administration, from 70 to 80 per cent of the daily oral dose is absorbed from the gastrointestinal tract; less than 0.5 per cent of the dose is excreted in the urine. When administration of the drug is discontinued, the plasma concentration falls slowly; within the first three days, 60 per cent of the drug disappears from the plasma, but 15 per cent is still present three weeks later. This suggests considerable localization in the tissues, although the concentration of drug in white blood cells is not high.

The drug is well tolerated in laboratory animals and in man; however, in one rat on a diet concentration of 0.5 per cent, autopsy showed a large yellow liver. In preliminary human toxicity studies, the drug was generally well tolerated; however one subject, after receiving a total of 17 grams in 2 gram daily doses, developed nausea, vomiting, malaise, fever and a generalized toxic reaction; all of these subsided without sequelae.

Early clinical trials with Camoform in human amebiasis have been underway in Latin America. In Peru, Barrios (1954), using an average total dose of 10.5 grams of Camoform treated twenty hospitalized individuals suffering from active amebic dysentery; he encountered no significant toxicity and he found that all were cleared of infection in a few days' time and that there were no relapses during follow-up periods varying from one to seven months. Payne (1954) treated over 900 patients during February and March of 1954 in an attempt to demonstrate whether Camoform would be effective in a one, two or three day treatment; it was established that one and two day treatments were not effective, and the three-day period only showed response in fifty per cent of the cases. Felsenfeld (1954), working in Jamaica, Southern Mexico and Costa Rica, found

[1] From the Medical Department of the Tela Railroad Co., United Fruit Co., La Lima, Honduras.

[2] The Camoform used in this study was supplied by the Department of Clinical Investigation of Parke, Davis & Co.

that a five-day treatment freed approximately 90 per cent of his patients of *Endamoeba histolytica*.

MATERIAL AND METHODS

Selection of patients. The only criterion for inclusion in this study was that there should be indisputable microscopic diagnosis of *E. histolytica* trophozoites or cysts. Ten of the twenty patients had acute dysentery attributable to the parasite, and ten were relatively asymptomatic at the time of Camoform administration.

Treatment schedule. All 18 adults received 0.50 grams three times a day for seven days, a total of 10.5 grams. A one-year-old child was given 0.75 grams daily for 7 days, and a 6-year-old child received 0.75 grams daily for only 3 days.

Race, sex and age. One patient was a North American; all of the others were Honduran mestizos. The ages ranged from one year to 57 years. Fourteen were males, six were females.

Criteria of cure. Post-treatment follow-up examinations began seven to ten days after completion of therapy. Most of the stool specimens were normally passed; one or two specimens from each patient were warm, liquid, obtained by purgation with two ounces of sodium sulfate. Apparent cure was admitted when a minimum of six negative specimens was obtained during a period of three to four months after treatment. All specimens were examined by both wet mount and iodine-eosin mount, and confirmed by slides stained by a modification of Kessel's hematoxylin method (Craig, 1948). Unfortunately, sigmoidoscopic examinations and cultures for *E. histolytica* could not be done.

RESULTS

Immediate response. Symptoms were rapidly alleviated in the dysenteric cases. Cramps, abdominal pain and tenesmus disappeared usually by the second day, and normally formed stools were achieved by the second to fifth day. *E. histolytica* was usually absent from the stools by the second day of treatment. However, one 49-year-old male (referred to as a failure, below) had only a partial response. The diarrhea and cramps of this man were controlled in two days; mucous, pus and blood disappeared in 4 days, but *E. histolytica* persisted in the stools during treatment and in the week following treatment.

Drug toxicity. There were no instances of nausea or vomiting in this series, nor other clinical evidence of drug toxicity. No effect was noted on the white blood cell count, red blood cell count, hemoglobin, urine, bromsulfthalein test, serum bilirubin or cephalin-cholesterol flocculation test.

Follow-up. During the follow-up period of three to four months, a minimum of six post-treatment specimens were examined on each patient, as detailed in the explanation of criteria of cure. Seventeen (85 per cent) of the twenty patients remained asymptomatic and free of the parasites; three (15 per cent) must be considered failures. The first of these failures, a 49-year-old male, had responded only partially to treatment; 4 weeks later he presented himself again with

bloody diarrhea and many *E. histolytica* in the stools. The second failure, a 51-year-old male, was an asymptomatic case; six post-treatment specimens were examined during the three weeks following treatment, and the last of these was again positive. The third failure, a 20-year-old girl who was an asymptomatic case, had a positive stool 5 weeks after treatment. The second and third failures responded to re-treatment with the same 10.5 gram schedule of Camoform.

COMMENT

The results (85 per cent apparent cure) in this small series are good, but not spectacular. Several of the currently used amebicidal drugs produce cure rates in this range. What commends it to further clinical investigation are these facts: (1) it is fairly effective in dysenteric as well as in asymptomatic cases; (2) it contains neither iodine nor arsenic, and seems to be very well tolerated in therapeutic quantities; (3) it can be produced economically.

Purposely, no effort has been made to differentiate sharply between trophozoite-passing individuals and cyst-passers. The omission was made in order to emphasize the fact that one should not speak of the treatment of cysts, as over against the treatment of trophozoites. All treatment is aimed at the ulcer-producing trophozoites; cysts do not cause pathogenesis. It is because the cysts develop from the trophozoites that their presence or absence can be used as an index of success or failure of the therapeutic procedure aimed at the trophozoite.

SUMMARY

A new synthetic amebicidal drug, Camoform has been described. Preliminary results indicate an apparent cure rate of 85 per cent among a small series of twenty individuals.

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THE EFFECT OF PUROMYCIN ON SIX SPECIES OF *TRYPANOSOMA* IN MICE

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In the past years there have been numerous reports indicating negative results on the use of antibiotics for the treatment of various trypanosome infections. However, recently two encouraging reports have been made, one by Hewitt *et al.* (1953) who reported a curative effect of Achromycin[1] (Puromycin) on *Trypanosoma equiperdum* infections in mice and rabbits, the second by McCowen *et al.* (1953) who stated that Erythromycin prevented death of mice infected with *T. equiperdum*.

Both antibiotics are produced from species of Actinomyces, *Streptomyces albo-niger* and *Streptomyces erythreus* respectively. The structure of Puromycin is reported by Walker *et al.* (1953), and its properties are described by Porter *et al.* (1952) who also report a fairly broad *in vitro* spectrum against bacteria.

Since the effect of a chemical agent on one species of trypanosome does not necessarily indicate the effect on other species we decided in our preliminary work to test the trypanocidal effect on as many species as possible. In this study we describe the effect of Puromycin on six trypanosome infections in mice.

METHODS AND MATERIALS

The following species of trypanosomes were used: *Trypanosoma equiperdum*, *T. equinum*, *T. evansi*, *T. gambiense*, *T. rhodesiense*, and *T. congolense*. All are strains which have been maintained in our laboratory by serial transfer in guinea pigs or rats for more than seven years.

Female white mice weighing approximately 20 grams were used as the host animal. Several passages were made through mice prior to the use of each strain. The mice to be infected were inoculated as one group and then divided into lots of 5 or 10 for the different categories of treatment. In each experiment, regardless of species, 10,000 trypanosomes were given intraperitoneally in 0.2 ml. of 0.85 per cent saline as inoculum. Puromycin was administered intraperitoneally in 0.2 ml. of saline. The drug was readily soluble, and the pH was not adjusted. Dosages of the drug used were based on information furnished by Hewitt *et al.* (1953).

Control animals were infected and not treated, and always died before treated animals except in a few cases when a relapse prolonged the life of a control, and in the case of *T. congolense* which did not prove fatal to the control mice during the time of the experiment. The course of the infection, the period of suppression following treatment, and the return of the parasites to the blood stream of un-

[1] Puromycin (originally called Achromycin) was obtained through the courtesy of Doctor R. W. Linton and Dr. R. I. Hewitt of Lederle Laboratories, American Cyanamid Company, Pearl River, New York.

cured mice were followed daily by microscopic examination of fresh drops of blood taken from the tail. Subinoculations were made from mice, except for those known to be positive, which survived the treatment of one or multiple doses more than 30 days. This was done by drawing as much blood as possible from each mouse by cardiac puncture and inoculating one or two fresh mice intraperitoneally. These, then, were examined for at least 30 days.

EXPERIMENTAL DATA

In preliminary tests, which are not tabulated in the tables, a total of 140 mg. per kg. of Puromycin was administered twice daily for 7 doses (20 mg. per kg. per dose), starting approximately 4 hours after inoculation. With this dosage 5 of the 6 trypanosome infections, *Trypanosoma equiperdum*, *T. equinum*, *T. evansi*, *T. gambiense*, and *T. rhodesiense* were prevented from developing in 5 of 5 mice each, and trypanosomes were not detected in any of the subinoculated mice during the 30 day period of observation. *T. congolense*, however, appeared in the blood stream 8 to 11 days after cessation of treatment in 4 of 5 mice. The fifth mouse remained negative but was not followed beyond the period of the experiment because the infection did not prove fatal to either control or treated mice.

Administration of the drug 4 days prior to inoculation with trypanosomes did not prevent infections from developing. A total of 140 mg. per kg. was administered in a single dose to each of five mice prior to inoculation with the six species of trypanosomes. All animals developed infections immediately and death occurred essentially as in the controls.

When infections were allowed to develop and treatment was begun at the height of infection some cures resulted as determined by microscopic examination and subinoculation to fresh mice as summarized in Tables 1 and 2. Table 1 gives the day treatment was begun, the parasitemia at that time, and survival data for the control and treated mice. The data on *T. congolense* were not tabulated since all mice relapsed with only one death occurring during the 45-day period of observation. A total of 140 mg. per kg. of the antibiotic was used against all six species of trypanosomes. It should be noted that considerably more negative survivors are recorded in the *evansi* group, *T. evansi*, *T. equiperdum* and *T. equinum*, than in the *brucei* group, *T. gambiense* and *T. rhodesiense*, regardless of single or multiple dose or total size of dose. Hewitt *et al.* (1953) reported a 20 per cent survival of mice infected with *T. equiperdum* when a dosage of 140 mg. per kg. was administered twice daily for 7 doses beginning near the crisis of the infection. Our survival rate was even greater. Four out of 5 mice did not relapse following either single or multiple dosage of a total of 140 mg. per kg. when treatment was begun on the fourth day following inoculation at which time parasitemia was rated as +++ (Tobie, von Brand, 1953).

The result with the same regimen of treatment against *T. evansi* was more striking though the 100 per cent curative effect might not hold with greater numbers of mice. Hewitt and his coworkers reported multiple doses as more effective and less toxic than single doses. This is in agreement with our data and is especially evident when the higher dosages were used.

TABLE 1

Survival in days of control mice and mice treated with Puromycin when treatment was initiated at the height of infection

Trypanosome Species	*gambiense*	*rhodesiense*	*evansi*	*equinum*	*equiperdum*
Parasitemia on (day) of treatment	+++ (4)	+++ (4)	+ to +++ (4)	− to +++ (6-7)	+++ (4)
Controls					
No. dead/* no. inoc.	10/10	15/15	5/5	10/10	5/5
Average survival (range)	4.2(4.0-4.5)	7.3(4.5-12.5)	5.5(5.0-6.0)	11.1(7.5-19.0)	5.6(4.5-7.0)
140 mg. per kg. total					
Single dose					
Negative† survivors	0	0	5	1	4
No. dead/* no. inoc.	5/5	10/10	0/5	9/10	1/5
Average survival (range)	15.7(13.5-17.5)	13.4(12.0-15.5)		29.2(22.5-36.5)	61.0
Multiple dose (BID 7)					
Negative† survivors	1	0	5	7	4
No. dead/* no. inoc.	4/5	10/10	0/5	3/10	1/5
Average survival (range)	15.5(14.5-16.5)	18.6(14.5-23.5)		39.3(35.5-47.0)	22.5
280 mg. per kg. total					
Single dose					
Negative† survivors		0		7	
No. dead/* no. inoc.		10/10		2/10‡	
Average survival (range)		13.5(12.5-17.0)		32.0(30.5-33.5)	
Multiple dose (BID 7)					
Negative† survivors		5		10	
No. dead/* no. inoc.		4/10‡		0/10	
Average survival (range)		30.4(28.0-33.5)			

	420 mg. per kg. total				
Single dose					
Negative† survivors		0		2	
No. dead/* no. inoc.		7/10‡		0/10‡	
Average survival (range)		17.0(13.5–21.5)			
Multiple dose (BID 7)					
Negative† survivors		3		9	
No. dead/* no. inoc.		3/10‡		0/10‡	
Average survival (range)		27.6(18.5–33.0)			

* = Dead due to trypanosomiasis.

† = Considered a negative survivor if no trypanosomes were found for at least 30 days following cessation of treatment.

‡ = Difference between negative survivors plus number dead from trypanosomiasis and the number inoculated are the number dead due to toxicity of Puromycin.

TABLE 2

Period of suppression in days following clearing trypanosomes from the blood stream

Trypanosome species	*gambiense*	*rhodesiense*	*evansi*	*equinum*	*equiperdum*	*congolense*
140 mg. per kg. total						
Single dose						
No. not relapsing	0	0	5	1*	4	0
No. relapsing/no. inoc.	5/5	10/10	0/5	9/10	1/5	5/5
Average suppression (range)	7.2(6.0–11.0)	4.6(3.0–5.0)		13.4(12.0–17.0)	10.0	3.4(2.0–5.0)
Multiple dose (BID 7)						
No. not relapsing	1*	0	5	7*	4	
No. relapsing/no. inoc.	4/5	10/10	0/5	3/10	1/5	
Average suppression (range)	8.0(7.0–9.0)	6.4(5.0–10.0)		22.3(21.0–25.0)	10.0	
280 mg. per kg. total						
Single dose						
No. not relapsing		0		7		
No. relapsing/no. inoc.		10/10		2/10†		
Average suppression (range)		5.3(5.0–8.0)		18.0(17.0–19.0)		
Multiple dose (BID 7)						
No. not relapsing		5*		10		
No. relapsing/no. inoc.		4/10†		0/10		
Average suppression (range)		18.5(17.0–21.0)				
420 mg. per kg. total						
Single dose						
No. not relapsing		0		2		
No. relapsing/no. inoc.		7/10†		0/10†		
Average suppression (range)		7.0(6.0–8.0)				

420 mg. per kg.—(*Concluded*)

Multiple dose (BID 7)						
No. not relapsing		3*		9		
No. relapsing/no. inoc.		3/10†		0/10†		
Average suppression (range)		19.3(12.0–23.0)				

* = Negative mice from which subinoculations were made to fresh mice.

† = Difference between number not relapsing plus number relapsing and number inoculated are the number dead due to toxicity of Puromycin.

Dosages of 280 mg. per kg. and 420 mg. per kg. were used against only one species from each group. *T. rhodesiense* and *T. equinum*. Table 1 shows the negative survivors with these higher doses when administered BID 7 and also indicates the deaths due to the toxic effects of the drug. In some cases the deaths may have been due to the combination of a high parasitemia plus the toxic effect of the drug, since the LD_{50} given by Hewitt *et al.* (1953) is 580 mg. per kg. This seems likely when one compares the number of premature deaths in *T. rhodesiense* and *T. equinum*, for the average parasitemia of the mice infected with *T. rhodesiense* was considerably higher than that of the mice infected with *T. equinum* when treatment was begun.

Table 2 gives data on the periods of incomplete suppression in those mice which relapsed following treatment started at the height of infection, as well as on those which did not relapse, thus indicating a curative effect. Again, it should be noted that there is a considerable difference in the suppressive effect between the two groups. The *evansi* group appears to be more sensitive to Puromycin than the *brucei* group. *T. congolense* was not sensitive enough for any cures to result from the dosage used, a total of 140 mg. per kg.

In Table 2 the surviving mice from which subinoculations were made to fresh mice are indicated. Trypanosomes were found in only one subinoculated mouse. This was in one infected with *T. equinum* from the group treated BID 7 with a total of 140 mg. per kg., and in this case one trypanosome had been found in the original treated mouse on the fifteenth day following cessation of treatment after which the bloodstream appeared free of parasites for the next 25 days. At this time the first subinoculation was made. Two additional transfers have been made, each after more than 30 days. None of the mice were found positive at any time. This does, however, show that there is danger of cryptic infections in animals which survive the 30-day period of observation and indicates that the original animal should probably be observed for a longer period of time. The one mouse infected with *T. equiperdum* which survived 61.0 days with a negative period of only 10.0 days also emphasizes this, for at times parasites were very difficult to find.

All the data presented in Table 1 and Table 2 are based on treatment of mice at the height of infection, usually day 4, in which case untreated controls began dying that day or the following day except in the case of *T. equinum* and *T. congolense*. With these two infections the low inoculum apparently caused slow development of the infection and relapses in the pattern of infection which accounts for the variation in parasitemia at the time treatment was begun.

SUMMARY

The antibiotic Puromycin produced from a species of Actinomyces, *Streptomyces albo-niger*, was tested for its trypanocidal properties against *Trypanosoma equiperdum*, *T. equinum*, *T. evansi*, *T. rhodesiense*, *T. gambiense*, and *T. congolense* infections in mice. The data presented in this report show that when treatment with Puromycin was begun approximately four hours after inoculation with these species of *Trypanosoma* all except *T. congolense* were prevented from devel-

oping. Administration of the drug four days prior to inoculation with trypanosomes did not prevent infections from progressing. When treatment was initiated at the height of infection the drug had a strong suppressive effect against all species except *T. congolense*. This effect was greater against those of the *evansi* group, *T. equiperdum*, *T. evansi* and *T. equinum*, than those of the *brucei* group, *T. rhodesiense* and *T. gambiense*. It is encouraging that this antibiotic has this powerful effect against these trypanosome infections.

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VARIATIONS IN THE DYE TEST FOR TOXOPLASMOSIS

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There is no question that the *in vitro* dye test of Sabin and Feldman (1948) is a very useful tool for the study of *Toxoplasma* infections. The results obtained in different laboratories in the United States have been, in general, consistent, as indicated by "blindfold" comparative tests. However, there are indications in reports from other countries that the test, still relatively unstandardized, may yield surprisingly low titers in cases of toxoplasmosis (Westphal, 1951). Standardization of the test has been attempted by Beverly and Beattie (1952) on the basis of the number of parasites present in the peritoneal exudates used in each particular run. In our own routine performance of the test in this Laboratory, we have encountered instances of interference or inhibition of the dye test reaction, the analysis of which has yielded information of considerable importance relative to standardization. It is the purpose of this paper to describe this phenomenon and present evidence bearing on its elucidation.

MATERIALS AND METHODS

In the routine performance of the dye test in this Laboratory, the procedure used is essentially the same as that described by Sabin, Eichenwald, Feldman and Jacobs (1952). The following additional details and modifications can be noted: we use, as the source for peritoneal exudate, mice infected intraperitoneally only three days earlier; exudates from 4-day or 5-day infected mice frequently show organisms which fail to stain in the negative controls, presumably because of their prior death. The exudate is removed from the mice by paracentesis. Secondly, the stock buffer for the buffered methylene blue solution is made up at pH 10.8, using Coleman soda-borax buffer tablets, formula #44. Third, the buffered methylene blue solution is prepared by adding 0.1 ml. of a saturated alcoholic solution of methylene blue to 1.5 ml. of the stock buffer. Then 0.1 ml. of this dye solution is added directly to each of the tubes in the test runs after incubation; this assures a sufficient quantity of buffer to produce the high pH necessary for rapid staining, and produces less floc than the more concentrated dye solutions added in smaller quantities. A number of different brands of methylene blue have been used without noticeable differences.

In the experiments to be reported here, various modifications in the dye test procedure were necessary. It will be best to present the details of these along with the description of the particular experiment. Where no modifications are described, the procedure was the routine one outlined above. In all instances in which comparisons were made, the same accessory factor was used. Accessory factor serum was stored at −50°F. in small quantities and thawed just prior to use in the tests. Storage was for no longer than two months.

OBSERVATIONS

Our first observations of failure of the dye test to give satisfactory results occurred in late July, August and September, 1951, after a period of almost two years of successful use of the procedure. The RH strain of *T. gondii* was in use. Known positive sera which in previous tests gave consistently clear-cut titers were used as controls. During the period mentioned, a number of failures were observed in the dye test. The low dilutions of the positive control sera failed to effect complete lack of staining of the toxoplasmas. Instead, even in dilutions above those in which a zonal effect might be expected but below the normal titer for the specimens, the percentage of stained parasites ranged from 25 to 50 per cent. The clear-cut change, at the usual dilutions of serum, from predominantly unstained to predominantly stained toxoplasmas was not discernible. Some factor seemed to be causing inhibition of the test reaction. In order to interpret the tests, it was necessary to take as the 50:50 endpoint that dilution of serum preceding the one in which the stained parasites appeared predominant, and for the positive control this endpoint was frequently much lower than usual. Examples of the results obtained in the "inhibited" tests are compared in Table 1 with other more sharply defined results with the same sera in "uninhibited" tests.

It can be seen that most of the "inhibited" tests showed a remarkable lack of progression compared to the clear readings in the "uninhibited" tests. The inhibition was not observed in all tests run during this period, indicating therefore that the failures were not due to deterioration of the positive control serum. It occurred so frequently, however, that it was not possible to be certain of the outcome of the runs of any particular day.

In an attempt to overcome these difficulties and also to find some explanation for them, it was decided to use another source of *Toxoplasma* for the production of the peritoneal exudate used in the test. This was not another strain but merely another line of the same strain with a somewhat different history. The new line, designated EK, is the original RH strain which was maintained by Dr. Carl Eklund of the Rocky Mountain Laboratory, U. S. Public Health Service, for some time before we received it through Dr. J. K. Frenkel. Simultaneous tests with the same serum specimens and with the EK and RH toxoplasmas yielded a very interesting and consistent difference. Typical examples of the results of comparative tests are shown in Table 2.

TABLE 1

Comparison of "inhibited" and "uninhibited" dye test results on the same serum specimens

Serum Dilution	Percentage of Stained Organisms Seen in							
	"Inhibited" tests				"Uninhibited" tests			
	E.J.	Sw.	Sa.	F.S.	E.J.	Sw.	Sa.	F.S.
0	—	—	—	—	0	—	—	—
16	42	29	56	49	—	0	0	0
64	32	36	48	33	0	31	0	0
256	22	65	33	73	54	17	0	35
1024	35	96	42	78	65	100	47	70

TABLE 2

Comparison of the response of RH and EK toxoplasmas in simultaneous runs of the in vitro *dye test*

Dilution of Test Serum	Percentage of Stained Toxoplasmas Seen in Test with					
	RH toxoplasmas			EK toxoplasmas		
	Serum #1	2	3	1	2	3
4	0*	—	73	0*	—	13
16	50	75	55	0	0	0
64	48	37	29	0	0	0
256	36	93	26	0	0	0
1024	24	100	34	0	71	0
4096	69	—	91	65	—	37

* The "0" figures represent a great preponderance of unstained parasites. Although a few stained organisms may have been present, counts were not made because on inspection the reaction was obviously positive, with only 1 or 2 per cent stained.

The difference between the results of the dye test performed with these two lines of *Toxoplasma* is immediately apparent. The tests run with the EK toxoplasmas gave clear-cut readings, while the percentages of stained organisms seen with the RH toxoplasmas made the test runs exceedingly difficult to interpret. The same findings were obtained in repeated comparative tests using known positive human and animal sera.

In order to see if these differences between RH and EK toxoplasmas might be related to the number of organisms present, several simultaneous tests with various dilutions of peritoneal fluid in accessory factor serum were set up. The results of one of these are shown in Table 3.

First of all, it is to be noted that the EK toxoplasmas used in these tests were very abundant in the peritoneal exudate. While no exact counts were made, the number of organisms exceeded 100 million per ml. of the exudate. On the other hand, the RH toxoplasmas were not as numerous; less than 30 million per ml. of exudate were present. It is obvious, also, that in this particular test the run with 1:5 ratio of peritoneal fluid to accessory factor serum in the EK test was not as clear-cut as those with ratios of 1:7, 1:9, and 1:10. In the latter, there was a definite progression in the proportions of stained and unstained parasites seen in the tubes with ascending dilutions of serum. The runs with the RH toxoplasmas were poor in all cases, but they were entirely negative with the lower dilutions of peritoneal exudate. Although dilution of the peritoneal exudate exerted an influence on the tests, this influence could not be correlated with the number of toxoplasmas, since the EK toxoplasmas were more numerous than the RH parasites and nevertheless exhibited little inhibition of the dye test reaction compared to the inhibition seen in the RH runs.

For routine performance of the dye test after these findings, the EK line of *Toxoplasma* and a second RH line derived from Frenkel were used without the recurrence of the inhibition phenomenon. A second opportunity for studying the phenomenon presented itself, however, when it reappeared in tests done by the

TABLE 3

Effect of dilution of peritoneal exudate in AC factor serum on dye test results

Serum Dilution	EK Strain* AC #5. Peritoneal Exudate: AC Factor in *Toxoplasma* Suspension			
	1:5 % stained	1:7 % stained	1:9 % stained	1:10 % stained
16	32	9	6	3
64	43	8	4	7
256	26	11	8	9
1024	49	35	45	45
Saline	96	100	95	96

Serum Dilution	RH strain† AC #5				
	1:2	1:4	1:6	1:8	1:10
16	88	65	74	32	43
64	>90	51	50	33	37
256	>90	63	47	27	35
1024	>90	92	75	42	67
Saline	99	98	98	92	98

* Very many toxoplasmas in original peritoneal exudate.
† Fewer toxoplasmas in original peritoneal exudate.

TABLE 4

Comparison of dye test results in tests of the same specimen set up simultaneously with exudate from CDC and NIH mice infected with the same line of RH toxoplasmas

Serum Dilution	Percentage of Stained Toxoplasmas Seen in Test with Exudate from			
	NIH mice		CDC mice	
Run #	1	2	1	2
0	0*	0	40	41
4	0	0	29	60
16	0	0	74	65
64	0	0	64	89
256	0	0	80	>90
1024	>90	>90	85	>90

* "0" indicates that the unstained parasites were so predominant that no count was made.

toxoplasmosis laboratory, Communicable Disease Center, Atlanta, Georgia, in the Spring of 1953. These tests were being done with the RH strain derived from this Laboratory. Dr. Marion Brooke of the Communicable Disease Center sent us mice infected with these parasites and thus afforded us the opportunity for a further comparative study. As can be seen in Table 4, the results were the same as those obtained earlier. Tests run with exudate from CDC mice gave essentially negative results, while preparations with infected mice from our own Laboratory (NIH) set up at the same time with the same accessory factor, gave clear-cut findings.

TABLE 5

Effect of dilution of accessory factor in saline on the dye test reaction

Dilution of Positive Serum	Percentage of Stained Toxoplasmas Seen in Test Run with Accessory Factor Dilutions								
	Undil	90% AC	80% AC	70% AC	60% AC	50% AC	40% AC	30% AC	20% AC
Undil.	0*	0	0	0	10	12	41	63	85
4	0	0	0	0	11	22	58	66	91
16	0	0	0	0	13	27	67	52	90
64	0	0	0	0	15	30	60	31	86
256	24	14	4	11	20	31	42	21	89
1024	78	48	17	52	32	75	68	51	93
4096	93	87	73	90	94	92	87	92	>95

Saline controls for all runs were 95% stained or higher.

* The "0" figures represent a great preponderance of stained parasites. Although a few stained organisms may have been present, counts were not made because on inspection the reaction was obviously positive, with only 1 or 2 per cent stained.

It was found, further, that the inhibitory phenomenon was not directly related to the number of parasites found in the CDC exudates. In some instances, satisfactory runs were obtained when large concentrations of parasites were present, while unsatisfactory runs were encountered when only small numbers were counted. It was felt, therefore, that some soluble component of the peritoneal exudate could be responsible.

In order to test for the inhibitory effect on the dye test of a soluble component of peritoneal exudate, it was necessary first to determine the extent to which accessory factor serum could be diluted without effect on the staining reaction. For this purpose a progressive series of AC-factor serum dilutions were prepared with normal saline. These were mixed in the usual manner with peritoneal exudate to make a 1:5 dilution of the latter, and test runs were set up with a known positive serum to see at what dilution the dye test reaction was not obtained. The results are shown in Table 5.

Although some small variations occurred in the endpoints of the runs with accessory factor dilutions up to 70 per cent, mostly limited to a 1-tube difference, these runs all showed complete lack of staining in the tubes with the lower dilutions of the positive serum. Beyond 70 per cent dilution of the accessory factor, although there was still a progression in the numbers of unstained and stained parasites seen in the ascending dilutions of the positive serum, the reactions were not as well-defined, in that even in the tubes with the greatest antibody concentration there were always found at least 10 per cent stained organisms. It was therefore decided that the safest maximum dilution of accessory factor which could be made in the dye test was 70 per cent.

For testing the inhibitory effect of soluble components on the dye test, it was then possible to dilute the accessory factor up to 70 per cent with such soluble components. Peritoneal exudate from 3-day infected mice was removed in the usual manner and 100 U.S.P. units of heparin per ml. were added to prevent clotting. It was then centrifuged at 2500 r.p.m. for 10 minutes to sediment the

TABLE 6

The inhibitory effect of soluble components of peritoneal exudate on the dye test reaction

Dilution of Positive Serum	Percentage of Stained Parasites Seen in Test Run with	
	70% AC / 30% supernate of exudate	70% AC / 30% saline
Undil.	96	0
4	90	0
16	82	0
64	84	0
256	93	15
1024	95	89
Sal.	95	98

TABLE 7

Titration of amount of soluble component of peritoneal exudate which interfered with the dye test reaction

Dilutions of Positive Serum	Percentage of Stained Parasites Seen in Test Runs in Which the Designated Percentage of Supernate of Peritoneal Exudate was Present in the Accessory Factor Serum Preparation*						
	0%	5%	10%	15%	20%	25%	30%
Undil.	0	0	0	0	27	63	93
4	0	0	0	20	60	79	>95
16	0	0	0	50	81	86	>95
64	0	0	45	70	85	92	>95
256	36	70	87	91	>95	>95	>95
1024	>95	95	>95	>95	>95	>95	>95
Saline	95	97	96	96	95	93	>95

* The percentage of AC factor was kept constant, at 70%, the difference in volume being made up with saline.

toxoplasmas and white cells, and the clear supernate was drawn off. For the test run, 0.3 ml. of this clear supernate was mixed with 0.7 ml. of accessory factor serum. A similar 70 per cent dilution of accessory factor serum in saline was used as control. *Toxoplasma* suspensions were then prepared by adding to each of the above two mixtures 0.3 ml. of fresh peritoneal exudate from other 3-day infected mice. Equal parts of these suspensions were mixed with dilutions of known positive serum and then incubated in the usual manner for the test procedure. The results of one such test are shown in Table 6.

Obviously, the addition of the supernate of peritoneal fluid to the dye test reaction mixture resulted in complete blocking of the reaction. This same result was found in repeated tests, and almost complete inhibition was also found when the accessory factor serum was diluted to only 80 per cent with the supernate. A titration of the amount of supernate which interfered with the dye test reaction is presented in Table 7. Even in those runs in which there was very little of the soluble component of peritoneal exudate present, there was some reduction in the dye test titer obtained with this positive serum, and the reduction was greater

with the progressively greater proportions of the component. The results of the runs with 15 per cent or more of the component present in the AC factor are similar to those seen in "inhibited" tests such as those described above.

CONCLUSION AND DISCUSSION

It is our conclusion from these experiments that the "inhibition" of the dye test recorded in the observations described above was due mainly to the presence of a soluble component of the peritoneal exudate of infected mice. It is believed that this component is soluble antigen, on the basis of reports by Sabin (1949) and by Bozicevich (personal communication) and Warren (personal communication) that peritoneal exudates freed of toxoplasmas and cells contain considerable complement fixing antigen.

In general, the concentration of soluble antigen present in the peritoneal exudates used is not sufficient to cloud the dye test reaction. Variation in the concentration of protein in the exudates, however, is known to occur, and it is probably in relation to such variations that the inhibition of the reaction is observed. Thus, Cathie and Dudgeon (1949) state that on some occasions the test fails to work for "no apparent reason." The factors involved in the production of high concentrations of antigen in the peritoneal exudates of mice infected with various strains of *Toxoplasma* are still to be elucidated.

It has been our practice, and we know it to be the practice of other workers, to accept only the results of tests in which the findings are clear-cut, with a sharp reversal from negative to positive. If the same base line is used by different laboratories, using as a starting point samples of the same positive serum, dye test results reported by these laboratories should differ by little more than a 1-tube dilution of the test serum, allowing for dilution differences. Thus, for example, the original positive control sera for our tests were furnished us by Dr. Harry Feldman, from proved cases of toxoplasmosis. Our results with these sera were the same as his, and subsequently used control sera were selected from tests in which these served as controls. Thus far, in a number of tests done by both laboratories, the results have been in good agreement. Similarly, Dr. Siim in Denmark and Dr. Feldman have obtained the same results in blindfold tests.

On the other hand, Westphal (1951) states that the titers he obtains are approximately ¼ as high as those obtained by American workers, and attributes the difference to the type of methylene blue used in the dye test. In Table 3 of his paper, the results he describes are similar to what we have designated above as an "inhibited" test. Such a test is extremely difficult to interpret, and it is felt that his lower titers are not due to the use of different brands of methylene blue so much as to the inhibition phenomenon. As stated above, we have used various brands of methylene blue without noticeable alterations in results.

In those cases in which inhibition of the dye test reaction occurs, it might be possible to produce a good test by first washing the toxoplasmas out of the exudate fluid. Such a procedure, used routinely, should result in better standardization of the dye test than current practice. The difficulty lies in maintaining the viability of the toxoplasmas during such manipulations. We have performed a

few tests to see if washing the parasites would be deleterious to them and have obtained satisfactory dye test results.

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SOME RECENT STUDIES IN BRAZILIAN PEMPHIGUS[1]

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A characteristic feature of dermatology of the New World is the endemic pemphigus of the central part of South America. Any studies relative to this striking type of skin reaction should be of interest to those in tropical medicine, for there are many problems of epidemiology, differential diagnosis and therapy, all related to dermatology in the tropics. Since this is the most extensive, most active, and perhaps only focus of endemic pemphigus in the world, there are still those outside of Brazil who doubt the relationship of the pemphigus of Brazil to pemphigus seen elsewhere in the world. Since no one knows what pemphigus is, one can argue only about clinical features. For those who actually work with the cases in Brazil, there is no doubt about the morphological relationships to pemphigus.

The classification of true pemphigus today is considered by Lever (1953) to be:

1) pemphigus vulgaris malignus
2) pemphigus vegetans
3) pemphigus foliaceus
4) pemphigus erythematosus (Senear-Usher)

Actually, however, because of the relationships of these, there are only two essential basic forms (Lever): pemphigus vulgaris malignus and pemphigus foliaceus.

Brazilian pemphigus begins usually with erythemato-squamous lesions on which develop sero-purulent bullous eruptions. These occur on the face and the anterior and posterior of the thorax in the seborrheic areas. The lesions become generalized. The febrile response depends on the severity of the lesions and the spread. With generalized lesions, the skin surface is moist under the extensive but superficial desquamation. Rubbing of the skin surface produces long sheetlike masses of skin scales with a moist surface underneath (phenomenon of epidermolysis or Nikolsky's sign). The popular name, *fogo selvagem* (savage fire), describes adequately a progressive development with bullae as extensive as those of a severe burn, and feelings of heat and severe discomfort. Various types of chronic skin changes and various visceral disturbances, including severe osteoporosis may result.

From the clinical studies at the present time (Vieira, 1940, 1948, 1951; Amêndola, 1947), there is justification for belief that the Brazilian type of pemphigus has certain characteristic features which are not found in pemphigus foliaceus described elsewhere in the world. These are in addition to the endemic feature

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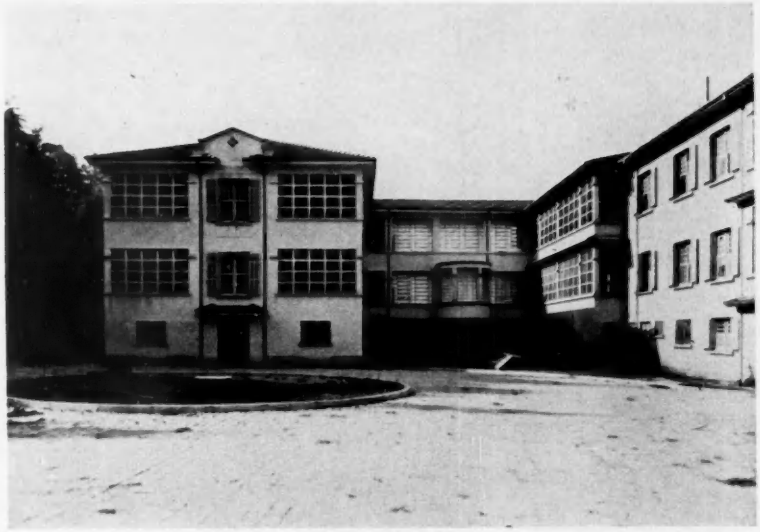

FIG. 1. The first hospital in the world solely for the care of pemphigus. Instituto Adhemar de Barros, São Paulo, Brasil. Has registered over 200 patients.

of Brazilian pemphigus. Maybe such distinctive features could be resolved in other areas if the frequency of this uncommon disorder was the same as it is in Brazil. These are:

1) frequency of pemphigus erythematosus (Senear-Usher type) as a precursor.
2) protracted febrile course—this may occur at times in pemphigus foliaceus elsewhere in the world.
3) extensive pyogenic infections.
4) frequent involvement of younger age groups.
5) involvement of the eye, especially cataract (Amêndola, 1947).
6) relatively better prognosis.

For the inexperienced observer, it sometimes appears strange that the erythrodermic phase, for example, seen for the first time, could be related to a preceding bullous disorder. The presence of a few bullae situated relatively superficially and the general moist character of the skin and the Nikolsky sign serve to differentiate this from ordinary exfoliative dermatitis. Detailed histologic studies reveal bullae, intra-epidermal in location, with features of acantholysis characteristic of the development of the lesion of true pemphigus. Such foci of acantholytic cells may be found even under heavy parakeratotic scaling. The Tzanck test demonstrates such acantholytic cells on the scraping from the early lesions. James Jambor in our department and Abilio Martins de Castro F. (1953) in São Paulo are examining such cytologic material under the phase contrast microscope.

The clinical features of Brazilian pemphigus include varying degrees of severity.

FIG. 2. The progressive stages of development of Brazilian pemphigus. A) Onset in September. B) One month later. C) Development of the generalized stage—three weeks—death shortly afterwards.

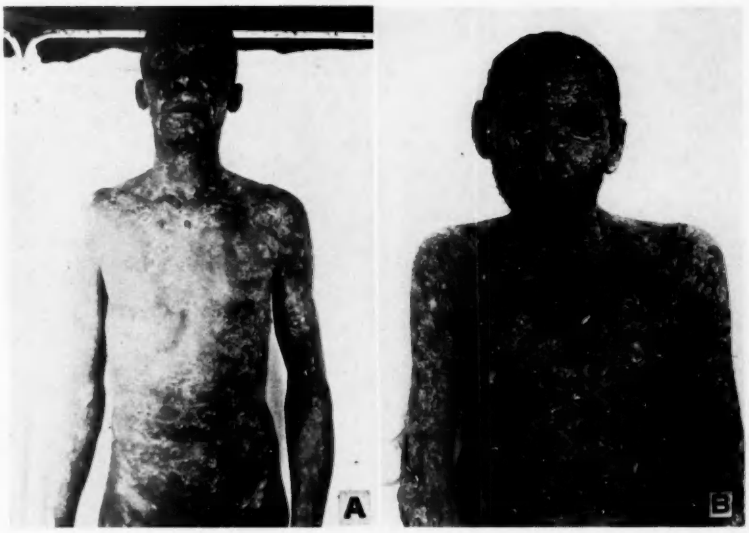

FIG. 3. A) Pemphigus foliaceus. Cincinnati General Hospital—black and white picture from Kodachrome. B) Pemphigus foliaceus—São Paulo, Brasil.

These may be classified briefly as acute, subacute (common) and chronic types. The latter comprise: "fruste"-abortive (Senear-Usher type: pemphigus erythematosus), bullous, typical foliaceus, erythrodermic, pustular, dystrophic, hyperpigmented, papillomatous and herpetiform.

For those interested in tropical dermatology it is of interest to note that this South American pemphigus has been considered variously as pellagra with severe secondary infection, drug eruptions, or "tropical pemphigus", so miscalled. "Tropical pemphigus" incidentally is not pemphigus at all but a type of pyoderma of the bullous impetigo group, and for that reason the term is unsatisfactory.

The diagnosis is made, then, by continued observation of the progress of the disease, the cytological findings on the Tzanck test and the corroborative histopathological features of acantholysis. With these characteristics in mind one can state quite simply that whatever pemphigus foliaceus may be, the Brazilian pemphigus is a type of pemphigus foliaceus.

Some features of epidemiology should be considered. The first references to this form in Brazil appears in 1891 (Campos, 1952). From 1929 to 1937, so many cases were found in the State of São Paulo that a special commission was established to study the disease. Extensive investigative and clinical studies were soon initiated by Vieira and his collaborators. The endemic zone as now determined appears still to be limited between the meridians 60° to 40° E. and parallels 15° to 24° S. This involves principally the southern portions of the states of Bahia, Goiás, Mato Grosso and São Paulo. Occasional small numbers have been

FIG. 4

FIG. 5

FIG. 4. Child with Brazilian pemphigus

FIG. 5. "Forme fruste" (Senear-Usher type) of Brazilian pemphigus—healed (BCG vaccine).

FIG. 6. Hyperkeratosis palmaris associated with chronic phase. Atabrine pigmentation also evident. Black and white photograph from Kodachrome.

found in Pernambuco, Amazonas and Espirito Santo and other areas. The frequency there may be considered the same as in other parts of the world.

There is no apparent decrease in the incidence of this disease. It is estimated that there are approximately 3,000 cases in the entire endemic zone. Many cases are not under medical observation but are kept hidden by the family, a type of cutaneous "skeleton in the closet." However, because of the extreme fear and anxiety about this disorder, arrested and healed cases remain usually

FIG. 7

FIG. 8

FIG. 7. Facies of hyperpigmented and papillomatous phase of chronic Brazilian pemphigus plus atabrine pigmentation. Black and white photograph from Kodachrome.

FIG. 8. Osteoporosis and contractural deformity of chronic Brazilian pemphigus. Patient of 36, with history of pemphigus for 22 years.

about the hospital rather than return to their community. There is also some evidence at the present time that the foci are expanding. The São Paulo focus is supposed to be responsible for the spread into the southern state of Paraná and also into Bolivia, Paraguay, and Argentina. The focus in Mato Grosso seems to have spread into Peru. No recent immigrants have acquired this disease in Brazil. There is apparently no characteristic racial distribution as in pemphigus vulgaris malignus in the United States, which "appears to be" more frequent in Jewish people. In one study in Brazil of the racial distribution in 1,500 cases, the majority of cases were in the Brazilians. The frequency of involvement in other racial groups in an area depends upon the distribution of those groups in that area. New cases continue to be found in both rural and urban communities. Vieira and Aranha Campos found malnutrition and a high incidence of intestinal parasitism in the background of patients with Brazilian pemphigus but no definite causal alimentary factor for the disease can be proved. Well nourished individuals and wealthy persons may acquire this disease.

All who have worked with Brazilian pemphigus believe this to be an infectious disease, although there seems to be no such concept for the various forms of true pemphigus in the United States. The opinion concerning the infectiousness of pemphigus in Brazil is based solely on clinical data, not confirmed as yet by laboratory experiments. It is supported by 1) the existence of definite endemic

zones; 2) the formation of a new focus by the transport of people from an infected zone into a non infected zone. (As a corollary, the removal of a small focus may help to clear an area; this has been found in Minas Gerais); 3) occasional familial incidence—rare in pemphigus elsewhere in the world.

Because of the belief in the infectious nature of the disease, it is even difficult to secure personnel for the care of these unfortunate patients in the pemphigus hospital. There is no known history of medical personnel, however, ever contracting the disease from their patients.

If Brazilian pemphigus is a form of true pemphigus then there is an extraordinary opportunity in this center of pemphigus to do concentrated investigative work, for as Lever (1953) states: "the cause of pemphigus is unknown and not even a convincing theory as to its cause exists." There are the usual speculations about the cause of pemphigus. These include bacterial and viral agents and even metabolic disturbances such as salt retention. There appears to be another factor which has been of interest to Kehoe (1948) and one of us (L. G.) for some time, chiefly through data furnished to us by Vieira over a period of years. The frequent occurrence of the erythrodermic phase, and the endemic foci in areas with mineral deposits, suggest that soil contamination with various types of heavy metals may be suspected. This speculation could be easily tested by detailed toxicological studies of patients in all phases of the disease and by spectrophotometric studies of the involved skin.

How is this disease of unknown etiology transferred in Brazil? Most of the experiments in regard to arthropod vectors have been done with the Simuliidae (Fonzari, 1945). As yet, the results are negative. It is indeed unfortunate that lack of funds has stopped these important studies on the apparent modes of transmission.

Special histochemical studies have also been initiated on prepared sections from different phases of pemphigus foliaceus. These studies include enzymes such as phosphates, lipases and acid mucopolysaccharides and also basic dyes (Atkinson, 1953). At present toluidine blue, pH 2.0, shows up the acantholytic areas well. Allochrome staining is a good contrast stain for study of the changes in the epidermis. Studies of the blood serum of these patients with pemphigus are also under way with the new spectrophotometric test of Cohen and Rein. This test measures the degree of light absorption of brom thymol blue (Rein, 1953).

The nonspecific therapy of Brazilian pemphigus is also of some interest to those in tropical medicine. Extensive secondary infection is often present. This infection is not only a serious complication for the patient but also a factor to be considered in prolonged corticotropin and corticosteroid therapies. Modern steroid therapy is of value in the treatment of pemphigus but the courses are of necessity long and the complications severe (Goldman *et al.*, 1952, 1953; Lever, 1951). Osteoporosis has been a particularly disturbing complication. Testosterone and estrogen, and high protein diet have been suggested by Kinsell, Boling, Partridge and Foreman (1953) to attempt to offset the catabolic activity of the steroids. Our attempts (Goldman *et al.*, 1953) at topical therapy of the pemphigus lesion with local applications of various forms of hydrocortisone acetate and free

alcohol have not been successful, probably because of the intense degree of local tissue change. More experiments are needed in the study of attempts at local inhibition of inflammation in the chronic phases of pemphigus foliaceus where the bullous activity may not be so great. We have just initiated a study of the cytology of the pemphigus bulla after local applications and local injections of various corticosteroids.

Because of the severity of the pustular phases mentioned above, antibiotics are needed. The extensive areas of involvement and the wealth of clinical material give opportunities for rapid clinical studies with antibiotics. Preliminary experiments with erythromycin appears to show some effect (Fonzari) though more detailed experiments are still needed. Erythromycin in combination with atabrine did not increase the activity of the atabrine (Fonzari). In a control series of superficial pyodermas such as impetigo, infected heat rashes and secondarily infected contact dermatitis, topical erythromycin has been effective (Goldman). In a group of deep pyodermas (furunculosis, nodular acne, erysipelas, hidrosadenitis axillaris), oral erythromycin has also been found effective (Goldman).

There is considerable interest at present in the effect of various antimalarials in the therapy of some chronic diseases of the skin. Atabrine (Fonzari, 1947, 1947a, 1948, 1948a, 1952) and now chloroquine (Goldman *et al.*, 1953) have been shown to have a definite effect in chronic forms of lupus erythematosus. Because of the necessity for prolonged courses in this disorder, the less toxic chloroquine is preferred although some investigators believe that atabrine acts more rapidly. Studies are now under way also with Plaquenil sulfate. With Fonzari's work, atabrine has been shown to be of value in Brazilian pemphigus while chloroquine in small dosage forms has not. The dosage of atabrine has varied, 0.60 to 0.30 grams daily. Although some reactions do occur, it is indeed curious that the drug can be given over prolonged periods of time, usually with none of the severe toxic reactions which we have observed in the therapy of malaria and in the therapy of discoid lupus erythematosus. Fonzari has observed only two cases of lichenoid atabrine dermatitis in 300 cases of Brazilian pemphigus treated over long periods of time. The atabrine pigmentation superimposed on a pemphigus foliaceus makes a striking picture. There is considerably difficulty evaluating visceral reactions to the drug since these, especially liver and renal involvement, can come from the pemphigus per se. Fonzari ranks atabrine secondary to corticotropin and corticosteroid in affecting the course of pemphigus foliaceus. Chloroquine does have some effect in Brazilian pemphigus especially as regards the exudation. It is believed inferior, however, to atabrine. Fonzari will repeat his studies with larger doses of chloroquine and with Plaquenil sulfate. The studies in the United States on the effect of chloroquine in pemphigus have just started. So far, we have no report of any rapid effects similar to that of corticotropin or corticosteroid. BCG vaccination has been used with success by Fonzari in the "forme fruste" (pemphigus erythematosus). The "nonspecific and effective reactions" of the antimalarials in various chronic dermatoses of unknown origin should be an interesting field of investigative work in modern dermatology. One

method in which we have been interested is the assay studies of these drugs in the skin. At present, Cole, Hughes, Schmidt and Goldman are studying levels of chloroquine in the serum and skin, including normal and involved skin.

CONCLUSIONS

Endemic pemphigus in the central part of South America with its extraordinary wealth of clinical material offers a place to meet the challenge of the perplexing riddle of pemphigus, for morphologically this severe cutaneous reaction is a type of true pemphigus. Detailed epidemiological studies, the factors making for the development of various clinical phases, the use of topical and systemic antibiotics, the mechanism of the local action of the corticosteroids and the study of the nonspecific action of various antimalarial drugs are but a few of the problems now under study.

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THE TRANSMISSION OF DENGUE BY *AEDES POLYNESIENSIS* MARKS

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Numerous epidemics of dengue have been reported from Melanesia and Polynesia south of the equator since early in the 19th century (Hirsch, 1883). Prior to World War II *Aedes aegypti* (Linn.) was the only proven vector of this virus known to occur in these areas. During World War II epidemiologic observations indicated that vectors other than *A. aegypti* might be responsible for the transmission of dengue in certain parts of Melanesia (Daggy, 1944 and Mackerras, 1946). As a result of these observations, experimental work was undertaken which demonstrated that *Aedes scutellaris* (Walker), a species widely distributed in Melanesia, could transmit the infection.

In the course of studies on the epidemiology of human filariasis on Tahiti from 1950 to 1952 the senior author noted that the local distribution of *A. aegypti* did not seem extensive enough to account for the widespread epidemic of dengue which had occurred on the island in 1944. Two species of mosquitoes, *Culex quinquefasciatus* Say and *Aedes polynesiensis* Marks, are widespread and abundant on Tahiti. The former species has been shown to be incapable of the biological transmission of dengue (Simmons *et al.*, 1931). The latter species, which is the most important vector of human filariasis (*Wuchereria bancrofti*) in Polynesia, has only recently been recognized as distinct (Marks, 1951) from the closely related mosquito, *Aedes pseudoscutellaris* (Theobald).

In 1953, an opportunity was afforded in the United States to test the ability of *A. polynesiensis* to transmit dengue using monkeys as vertebrate hosts. Since dengue infections in monkeys cannot be recognized by clinical manifestations, serological methods were employed to establish their occurrence.

MATERIALS AND METHODS

Virus. One ml. of human serum containing the Hawaiian strain of dengue virus which had never been passed in mice was inoculated intraperitoneally into

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the monkey which served as the donor for all mosquito feedings. Prior to its use this serum had been stored in the frozen state for 5 weeks in a box containing solid CO_2. Titration in suckling mice indicated that the serum had at least $10^{5.5}$ infective doses of virus per ml.

Monkeys. Two rhesus monkeys (*Macaca mulatta*), Nos. 1 and 2, and two cynomolgus monkeys (*Macaca irus* group) from the Philippine Islands, Nos. 488 and 490, were used in these experiments. Monkey No. 490 was used as the donor. These animals were housed in a mosquito-free area in a building separate from that in which the mosquito colonies were maintained. Prior to their use, the sera of these monkeys were tested for the two immunologic types of dengue antibody and none was found.

Mosquitoes. The *A. polynesiensis* used in these experiments were from a laboratory colony which was started with specimens from the Samoa Islands and which is maintained at the School of Hygiene and Public Health, Johns Hopkins University. Before specimens were selected for these experiments the colony was carefully checked to be sure that it was not contaminated with *A. aegypti* or any other species of mosquito. In addition, each mosquito used was identified at the time that it fed on the donor monkey and also at the time that it fed on the recipient monkey.

The mosquitoes fed readily when the shaved leg of an anesthetized monkey was inserted into the cage in which they were held. The engorged specimens were placed in cylindrical, pint-sized ice cream cartons, the tops and bottoms of which had been replaced by bobbinet. These cartons were kept in a constant temperature incubator in which the dry bulb temperature was maintained at 79° to 80°F. A high relative humidity was assured by placing the cartons on pads of moist cotton. Except for the experimental blood meals, the mosquitoes were fed only on apple slices.

Serological procedures. Serial dilutions of sera obtained from each monkey prior to and subsequent to the experimental feedings of mosquitoes were tested for the presence of hemagglutination-inhibition antibodies against 16 units of dengue hemagglutinin of the Hawaiian type. The recovery and characteristics of hemagglutinins from the two types of dengue virus have recently been reported elsewhere (Sweet *et al.*, 1953).

RESULTS

One lot of *A. polynesiensis* was fed on the donor monkey 4 days after inoculation of the dengue virus, another lot was fed 6 days afterwards, and a third lot was fed 8 days afterwards. These three lots of mosquitoes, after being held for extrinsic incubation periods of 16 days, 14 days, and 12 days respectively, were subsequently fed on three recipient monkeys. The number of specimens of each lot which fed on each of the recipient monkeys is given in Table 1.

The hemagglutination-inhibition titers of the sera of the donor and recipient monkeys are given in Table 2. It will be noted that all monkeys developed antibody indicative of dengue infection.

TABLE 1

The number of A. polynesiensis *of each lot which fed on the three recipient monkeys*

Mosquito Lot No.	Days After Inoculation of Virus Fed on Donor Monkey	Extrinsic Incubation Period	No. of Mosquitoes Fed on Indicated Recipient Monkey		
			Cynomolgus 488	Rhesus 1	Rhesus 2
1	4	16	23	35	31
2	6	14	33	50	40
3	8	12	26	25	30
Total mosquitoes fed on each monkey.....			82	110	101

TABLE 2

Hemagglutination-inhibition titers of sera of the donor and recipient monkeys against 16 units of Hawaiian dengue hemagglutinin

Monkey No.	Mode of Infection	Titer of Antibody at Indicated Time		
		Before use	36 days	59 days
Cynomolgus 490....	Human serum intraperitoneally	<10	—	80*
Cynomolgus 488....	Mosquitoes	<10	20	20
Rhesus 1.....	Mosquitoes	<10	80	—
Rhesus 2.....	Mosquitoes	<10	>1280	640

* This specimen was obtained at 56 days.

DISCUSSION

The demonstration that *A. polynesiensis* is capable of transmitting dengue is not surprising in view of the taxonomic relationship of this species to the other proven vectors, *A. aegypti*, *Aedes albopictus* (Skuse), and *A. scutellaris*. All are members of the subgenus Stegomyia. In addition, *A. polynesiensis* belongs to the *scutellaris* group of this subgenus as does *A. scutellaris*. Inasmuch as the *scutellaris* complex, which now includes 17 species distributed throughout the Australian Region and the eastern part of the Oriental Region, is a very homogenous group, it is not unlikely that others of its members are also capable of transmitting this virus.

Both rhesus and cynomolgus macaques have previously been shown to be susceptible to infection with dengue (Blanc *et al.*, 1929; Simmons *et al.*, 1931; Findlay, 1932). Since these primates occur in endemic areas of the human infection, it has been suggested (Simmons *et al.*, 1931) that they might have a role in the epidemiology of the infection similar to that subsequently shown for the lower primates in the epidemiology of yellow fever. If such is the case, mosquito vectors which occur both in the bush and around human habitations, such as *A. albopictus* and members of the *scutellaris* group, would be especially important in the maintenance of the transmission chain.

In addition to the observations for Tahiti, there are data which suggest that *A. polynesiensis* is an important vector of dengue in other widely separated parts of Polynesia. An extensive epidemic of dengue was reported from Manua Islands in American Samoa (Hargrave, 1931). A recent mosquito survey of these islands showed that the only species of *Aedes* present is *A. polynesiensis* (Jachowski, 1953). Similarly, a widespread epidemic of dengue occurred in the Marquesas Islands (Mumford and Adamson, 1944). Again, a recent survey by the senior author (Stone and Rosen, 1953) indicated that the only species of *Aedes* present is *A. polynesiensis*. While it is possible that *A. aegypti* existed in these areas at the time of the dengue epidemics and subsequently disappeared spontaneously, such an event is unlikely. Finally, it was reported (Amos, 1947) that the predominant mosquito in the neighborhood of each outbreak of dengue in Suva, Fiji Islands, in 1943 was a member of the *scutellaris* group (the three species of this group including *A. polynesiensis*, now known to occur in the Fiji Islands, were not recognized as distinct at that time). *A. aegypti* was present in the area but was very rare.

The biology and control of *A. polynesiensis* is similar to that of *A. scutellaris* described by Mackerras (1946). Both species are more difficult to control than *A. aegypti*.

SUMMARY

Epidemiologic observations in Polynesia suggested that *Aedes polynesiensis* Marks served as a natural vector of dengue in that area. This mosquito species was shown to be capable of transmitting dengue from monkey to monkey in the laboratory. A hemagglutination-inhibition test was used to demonstrate the occurrence of the infection in the experimental monkeys.

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MURINE TYPHUS FEVER IN SOUTHWEST GEORGIA, JANUARY 1945–JANUARY 1953

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An opportunity for further study of the epidemiology of murine typhus fever was afforded when 452 cases were intensively studied as part of an evaluation of a county-wide DDT dusting operation on the incidence of typhus fever. Results of this dusting program and epidemiological data pertinent to its evaluation have been reported by Hill *et al.* (1948) (1951).

The area in this study included Brooks, Grady, Thomas and Decatur Counties in southwest Georgia. These counties were selected because of the reported high endemicity of typhus fever and the similarity of population distribution between them. Cases were located by reports from State and local health departments, from hospital and laboratory records, and by frequent periodic visits to physicians practicing in the area. A clinical and an epidemiological history and blood specimens were obtained from each reported case and these data were evaluated. Final confirmation of a case was based on a positive complement fixation test at a titer of 1:4 or better.

SEASONAL DISTRIBUTION

Cases occurred every month of the year but reached a seasonal peak during the summer months as shown in Figure 1. This finding was similar to the distribution reported by Maxcy (1926) and Bowdoin *et al.* (1940), and was one of the main points used by Maxcy in differentiating this disease from epidemic typhus fever. However, when cases are divided by place of residence, the seasonal peak occurs during the summer in rural areas, but cases are fairly evenly distributed throughout the year in urban areas. This difference in seasonal occurrence of typhus fever between rural and urban areas can be explained by differences in chances of exposure to rats and rat fleas. Hill *et al.* (1948) and Rumreich *et al.* (1945) found a positive correlation between seasonal occurrence of typhus fever and seasonal prevalence of the oriental rat flea, *Xenopsylla cheopis*. Along with this, farmers are more exposed to rats and rat fleas in the summer when they have used up most of their stored grains from the previous year's harvest. In urban areas, the number of fleas on rats is related to temperatures maintained in buildings and does not have a wide seasonal variation so the chances of exposure to rats and rat fleas are more randomized throughout the year than on farms.

INCIDENCE

Table 1 gives the incidence of typhus by rural and urban areas. All cases which occurred in towns of less than 1,500 population or on farms were considered as

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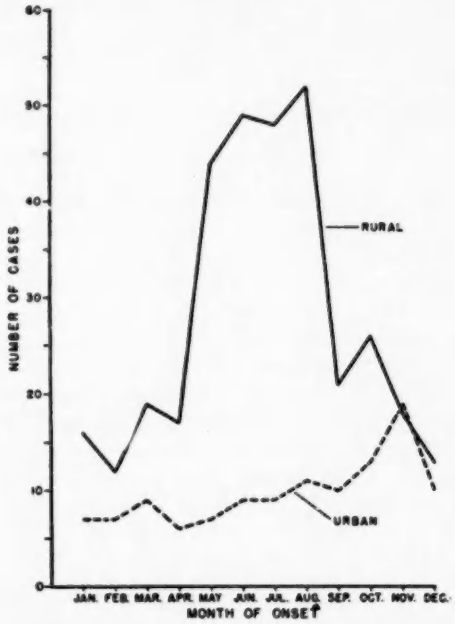

FIG. 1. Seasonal distribution of 452 cases of murine typhus fever by urban and rural areas in 4 southwest Georgia counties, January 1945–January 1953.

TABLE 1

Incidence rate per 1,000 for murine typhus fever by rural, urban, and total areas, January 1945–January 1953

	Population*	Cases	Rate
Rural	47,556	335	7.0
Urban	35,231	117	3.3
Total	82,787	452	5.4

* Adjusted July 1, 1948.

rural. The incidence was found to be about twice as great in rural areas as in urban areas. This is contrary to the urban-rural distribution found by Maxcy (1926), Beck *et al.* (1950) and Bowdoin *et al.* (1940). Current literature has indicated that the general concept of the urban-rural distribution of this disease has undergone a change. Early studies by Maxcy (1926) and Rumreich *et al.* (1931) emphasized that typhus fever was mainly an urban disease while later reports have pointed out that there has been an extension into rural areas (Topping *et al.*, 1943). Difficulty in evaluating the apparent change in the urban-rural distribution of this disease results from several things: different methods

TABLE 2

Incidence rate per 1,000 for murine typhus fever by sex and race, January 1945–January 1953

Race	Male			Female			Total		
	Pop.†	Cases	Rate*	Pop.	Cases	Rate*	Pop.	Cases	Rate*
Colored	16,469	24	1.4	18,258	14	0.8	34,727	38	1.1
White	24,164	249	10.3	23,896	165	6.9	48,060	414	8.6
Total	40,633	273	6.7	42,154	179	4.2	82,787	452	5.4

* Rate/1000.

† Adjusted to July 1, 1948.

used in case-finding in various studies, the fact that most typhus control measures have been carried on in the cities, different definitions of what constitutes rural and urban areas, and changes in medical care extended to rural areas.

The incidence rates per 1,000 by sex and race are given in Table 2. In general, the incidence by sex and race was found to be quite consistent with those rates reported in the literature (Maxcy, 1926; Bowdoin *et al.*, 1940; Beck *et al.*, 1950). Rates for males were higher than those for females and the rate for the white race was about eight times that found for the colored race. Considering the mode of acquiring typhus fever, differences in incidence rates by sex can be explained on chances of exposure. The peculiar distribution by race has not been explained but has been reported several times in other studies (Maxcy, 1926; Bowdoin *et al.*, 1940; Beck *et al.*, 1950). Maxcy (1926, 1929) was unable to explain it by a difference in clinical appearance of the disease in the colored race. One report in the literature contradicts this difference in incidence rates reported for the white and colored races. Rickard (1951) did complement-fixation tests for typhus fever on 3,678 serum specimens submitted for premarital syphilis examination and found the number of positives for typhus fever slightly higher in the colored than in the white race.

Figure 2 shows the distribution of cases by age groups. The incidence by age groups is low in childhood and then rises during the second and third decades to a peak incidence during the fourth and fifth decades. Bowdoin *et al.* (1940) have previously pointed out that age-specific incidence rates demonstrate there is no age-specific immunity in this disease.

OCCUPATION

The distribution of cases by occupation is given in Table 3. Farmers, farm wives and farm children made up 70 per cent of the cases studied, which serves to illustrate again the rural predominance of this disease and points out the occupation which is chiefly at risk.

LABORATORY RESULTS

Most of the cases in this series had two or more Weil-Felix and complement fixation tests done on serum specimens. A comparison of mean titers of the Weil-Felix and complement fixation tests, found by weeks from the date of onset of

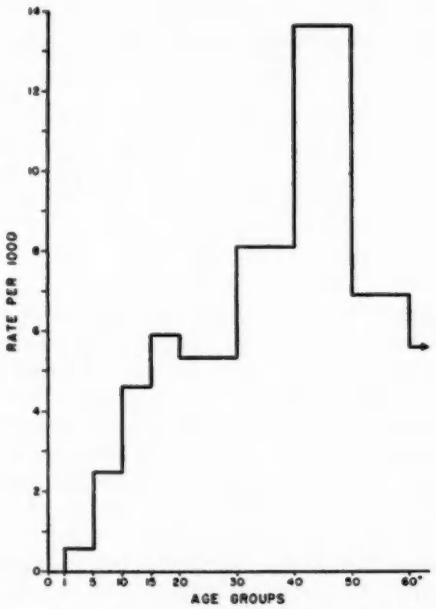

FIG. 2. Typhus fever incidence rate per 1,000 persons by age groups. Based on study of 452 cases in 4 southwest Georgia counties, January 1945–January 1953.

TABLE 3

Distribution by occupation of 452 cases of murine typhus fever in Southwest Georgia, January 1945–January 1953

Occupation	Number of Cases	Per Cent of Total Cases
Farmer	145	73.2
Rural housewife	82	
Rural children	57	
Food handlers	47	
Professional	13	
Others	108	26.8

illness, is given in Figure 3. The mean titer of the Weil-Felix test was high in the first week and then rapidly declined to a mean titer of 1:100 by the tenth week. The mean titer of the complement fixation test reached a peak during the second week and remained high over the next 50–60 weeks after onset of illness; then it slowly declined over the next 6 months. The length of time between onset of typhus fever and development of positive Weil-Felix and complement fixation tests could not be determined in this series. However, Scoville, as reported by

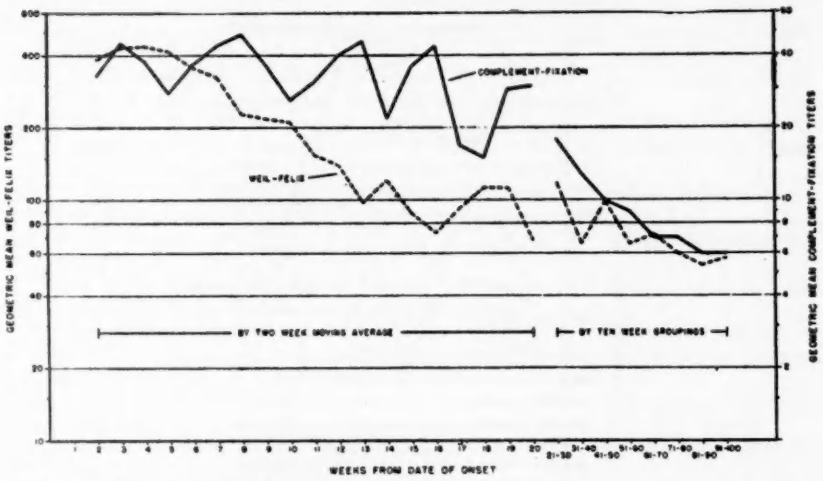

FIG. 3. Mean titers of Weil-Felix and complement-fixation tests in 452 cases of typhus fever by weeks from date of onset.

Smadel (1948), followed the course of serological response in 15 untreated cases of murine typhus fever, in which antibodies for the Weil-Felix reaction developed rapidly after onset of illness while antibodies for the complement fixation test developed more slowly.

EFFECT OF AUREOMYCIN ON WEIL-FELIX AND COMPLEMENT FIXATION TITERS

Antibiotic therapy of rickettsial diseases has been reported to change the pattern of antibody response. This effect was recently reviewed by Schubert (1952). The Weil-Felix test detected antibodies in rickettsial infections in treated patients but showed lower titers, whereas results of the complement fixation test in treated patients were more variable and appeared to depend on the interval of time between onset of illness and initiation of antibiotic therapy. If antibiotics were given before the third day of illness, there was little or no response of complement-fixing antibodies and if they did appear, the time of development was delayed.

To demonstrate the effect of aureomycin on the Weil-Felix and the complement fixation tests, a comparison was made of mean titers of these tests on all blood samples drawn from 0–10 weeks from onset of illness in cases treated and not treated with aureomycin. The results are shown in Figure 4. The majority of the treated cases received aureomycin within 3–5 days after onset of illness. The mean titer of the complement fixation test in treated cases was consistently lower than that in untreated cases and appeared to fall off more rapidly. While there appeared to be a slight delay in response of antibodies as demonstrated by the Weil-Felix test in treated cases, there was no consistent lowering of mean titers over the 10-week period.

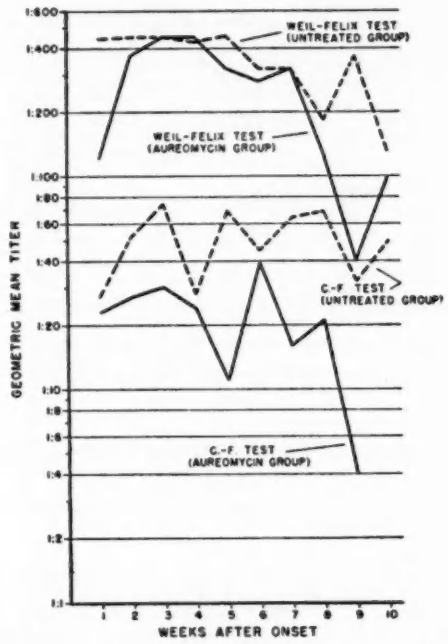

FIG. 4. Effect of aureomycin on the Weil-Felix and complement-fixation titers in murine typhus fever.

SUMMARY

A study of 452 cases of murine typhus fever occurring in Southwest Georgia from January, 1945, to January, 1953, showed a seasonal peak of rural cases during the summer but an even distribution of urban cases throughout the year. Incidence rates were about twice as high in rural as in urban areas, and about eight times greater in the white than in the colored race. Age-specific incidence rates were low in childhood, rising to a peak during the fourth and fifth decades. Farmers and their families made up 70 per cent of the cases studied. The mean titer of the Weil-Felix test was high in the first week and then rapidly declined, whereas the mean titer of the complement fixation test reached a peak during the second week and remained high over the next 50–60 weeks after which it slowly declined. In cases treated with aureomycin, there was a consistent lowering of mean titers for the complement fixation test, but very little effect on the Weil-Felix titers was demonstrated.

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TRIATOMINAE IN EXPERIMENTAL TRANSMISSION OF PLAGUE[1]

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Triatominae are found in large numbers in nature in lodges of the wood-rat, *Neotoma* spp. (Wood, 1945), with other insects. The wood-rat has been implicated in sylvatic plague (Meyer, 1942; Link, 1949), and its fleas have been shown to transmit plague (Eskey and Haas, 1939; 1940). Little work, however, has been conducted on the possibility of Triatominae as vectors of plague.

Mertens (1938) demonstrated that *Triatoma rubrofasciata* (De Geer) is capable of harboring *Pasteurella pestis* for a period of 30 days by injecting a pulverized suspension of the insects into guinea pigs. However, he did not find any plague bacilli in the excreta nor in the salivary glands of the infected insects after 72 hours. He fed 12 plague-infected *T. rubrofasciata* to 4 young rats; the rats did not contract plague. Attempted host-to-host transmission also gave negative results, i.e., allowing the *Triatoma* to feed on an infected guinea pig and then transferring the *Triatoma* to a non-infected host. No transovarial passage of plague bacillus has been demonstrated from infected Triatominae to their progeny.

Plague transmission studies using mice and guinea pigs as laboratory animals and 8 species of Triatominae as experimental insect vectors are reported here. Phenomena considered are: (1) the time period for which Triatominae could retain virulent plague bacilli; (2) the possible transmission of plague by various species of Triatominae through feeding; (3) the possibility of plague transmission through ingestion of infected insects by the mammalian host; (4) the possible presence of infectious *P. pestis* in the feces of infected Triatominae.

The experiments described in this paper are preliminary in nature and a more comprehensive study is in progress.

MATERIALS AND METHODS

Triatominae used in these studies were: *Triatoma protracta protracta* (Uhler), *T. dimidiata* (Latreille), *T. recurva* (Stål) (= *T. longipes* Barber), *T. phyllosoma pallidipennis* (Stål), *T. rubida uhleri* (Neiva), *T. platensis* Neiva, *T. infestans* (Klug), and *Mestor megistus* (Burmeister). These species were obtained from colonies maintained in the Department of Entomology, School of Tropical and Preventive Medicine, College of Medical Evangelists, Loma Linda, California.

[1] These studies were aided in part by a grant (E-173) from the National Institutes of Health, U. S. Public Health Service, Department of Health, Education, and Welfare; and were conducted in Loma Linda and San Francisco.

This paper is part of a thesis submitted by Charles T. Ames to the faculty of Walla Walla College, Washington, in partial fulfillment of the requirements for the Master of Arts degree in Zoology.

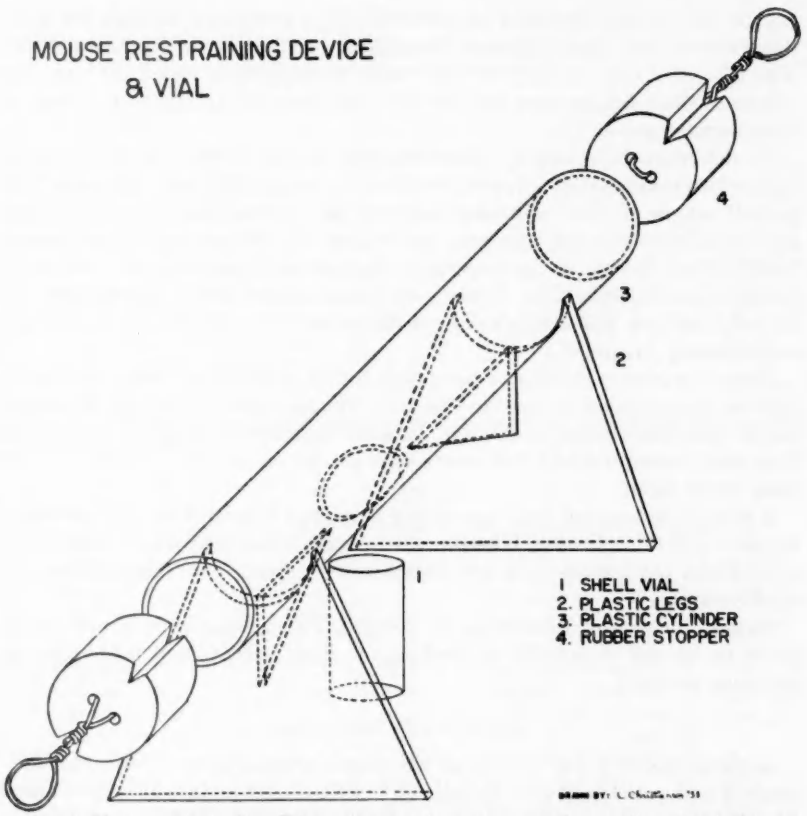

FIG. 1. Unit used in feeding Triatominae in plague transmission studies. Shell vial was held in place against abdomen of mouse with masking tape.

A virulent Indian strain of *Pasteurella pestis* (195/p) obtained from the George Williams Hooper Foundation, laboratory albino Swiss mice (Princeton strain), and guinea pigs were used.

The method used to infect the Triatominae was similar to that followed by Burroughs (1947) to infect fleas. Briefly, it consists of infecting mice by intraperitoneal inoculation with a dilution of virulent plague broth culture. Forty-eight hours later, when bacteremia in the mouse becomes sufficiently intense as determined by microscopic examination of blood smears, the animal is restrained and the insects allowed to feed on the infected animal.

The mouse was restrained in a device similar to that developed by Link and Prince (1950) and is shown in Figure 1. The Triatominae were confined singly, unless otherwise stated, in a shell vial slightly smaller in diameter than the central opening of the mouse restraining tube. Nylon gauze over the open end of

the vial imprisoned the insect but permitted it to feed freely through the gauze when the vial was placed against the abdomen of the mouse (Ryckman, 1952). The tube was held securely in place with masking tape during feeding. The insects in these studies were kept at 30°C. and were fed usually once during the experimental period.

To determine how long *P. pestis* remained virulent within the Triatominae, bugs which consumed an infected blood meal were sacrificed and triturated with a small volume of sterile physiological saline. Mice and guinea pigs were injected subcutaneously with the triturate; each mouse and guinea pig received respectively 0.2 and 0.5 ml. of the suspension. Animals which subsequently died were autopsied and examined for *P. pestis* by tissue smears, animal inoculation, and bacterial culture. Those which did not die within 10 to 14 days were sacrificed and similarly examined.

Forty-five insects which had previously fed on infected mice were allowed to feed on normal mice to test the ability of Triatominae to transmit the plague bacilli from one animal to another. In most instances feeding on the infected host was interrupted and the insects were placed on normal mice to complete their blood meal.

A total of 66 infected bugs were fed to 23 mice. The mice were confined singly in quart jars (Douglas and Wheeler, 1941) without food and water for 18 hours after which the insects were introduced into the jars. The Triatominae were readily eaten.

Sixteen samples of feces from 31 infected Triatominae were tested for *P. pestis* by animal inoculation as well as by direct smears and bacteriological culturing methods.

RESULTS AND DISCUSSION

In these studies it was found that the plague organisms survived in the Triatominae and were infective to animals up to 3 days after an infectious blood meal (See Tables 1 and 3). Differences in results from those of Mertens were possibly due to a difference in temperature and humidity under which the insects were maintained, frequency of feeding the insects, or other unknown factors. It is possible that there is a species difference in the ability of Triatominae to harbor *P. pestis*.

The data presented in Table 3 show that *Triatoma protracta*, *Triatoma infestans* and *Mestor megistus* remained infected for 3 days, but were negative on the fifth day.

Plague transmission by bite was accomplished only by *T. phyllosoma*. *T. phyllosoma*, by comparison with other species, is a large bug and a slow feeder and may be better suited to transfer *P. pestis* from an infected to an uninfected host because of the larger size of its mouth parts and greater length of time required to obtain a blood meal. This method of transmission would not seem important in the epizootiology of plague in nature because wood-rats are usually solitary dwellers, and are the preferred hosts of Triatominae in the Southwestern United States.

TABLE 1

Results of inoculation of mice and guinea pigs with triturated Triatoma protracta *which had previously fed on plague-infected mice*

*TIME INTERVAL	PRESENCE OF PLAGUE
Immediate	+
12 hours	+
24 hours	+
36 hours	+
48 hours	+
48 hours	+
48 hours	−
60 hours	+
72 hours	+
5 days	−
7 days	−

* Interval between infection and inoculation of Triatominae. Two insects were used at each time interval.

TABLE 2

Results of interrupted feeding trials with Triatominae and laboratory mice

TRIATOMA SPP.	NUMBER INSECTS	NUMBER MICE	*TIME INTERVAL	PLAGUE TRANSMISSION
T. protracta	20	3	Immediate transfer	−
T. rubida	4	2	Immediate transfer	−
T. lecticularius	3	1	Immediate transfer	−
T. dimidiata	1	1	Immediate transfer	−
T. infestans	4	1	Immediate transfer	−
T. phyllosoma	2	1	Immediate transfer	+
T. phyllosoma	2	1	Immediate transfer	+
T. protracta	4	2	8–10 hours	−
T. rubida	1	1	12 hours	−
T. protracta	4	2	6 days	−

* Interval between blood meal on infected mouse and on normal mouse.

Ryckman (unpublished data) has found that *Neotoma* readily devour cone-nosed bugs. Field observations seem to indicate that wood-rats also readily eat many Triatominae under natural conditions. If these Triatominae occasionally feed on plague-infected wood-rats, such an insectivorous habit could serve as a means of transmission of *Pasteurella pestis*.

Of 13 attempts to ascertain whether plague could be transmitted to susceptible laboratory mice by ingestion of infected Triatominae, 3 mice proved positive with primary pneumonic plague lesions. This result indicates that infection was initiated through accidental aspiration of infected material into the lungs and that no infection occurred by penetration of the gastrointestinal tract. The negative result is readily understandable in view of the data obtained by Quan (1953) who found that plague infection through the gastrointestinal tract re-

TABLE 3

Results of ingestion of plague-infected Triatominae by laboratory mice

TRIATOMINAE	NUMBER INSECTS	*TIME INTERVAL	NUMBER MICE	PLAGUE INFECTION
T. protracta	11	Immediate	2	−
T. protracta†	4	Immediate	1	+
T. protracta†	4	Immediate	1	+
T. protracta†	3	Immediate	3	−
T. infestans†	1	Immediate	1	+
T. infestans	5	Immediate	1	−
T. platensis	2	Immediate	1	−
M. megistus†	4	Immediate	2	−
M. megistus†	2	24 hours	1	+
T. protracta	2	24 hours	1	−
T. protracta	2	24 hours	1	+
T. protracta	4	48 hours	1	−
T. rubida	4	48 hours	1	−
T. protracta	3	72 hours	1	+
T. protracta	3	72 hours	1	+
T. protracta	9	72 hours	3	−
T. protracta‡	2	7 days	2	−

* Interval between infectious blood meal and ingestion of Triatominae.

† These Triatominae were known to be infected with *Trypanosoma cruzi* as well as with *Pasturella pestis*.

‡ These Triatominae were triturated with normal saline and given to the mice by oral gavage.

TABLE 4

Tests for virulent Pasturella pestis *in Triatominae feces following an infective blood meal*

TRIATOMA SPP.	NUMBER INSECTS	NUMBER MICE	*TIME INTERVAL	PLAGUE ISOLATION
T. protracta	10	5	within 15 minutes	−
T. platensis	2	1	within 15 minutes	+
T. dimidiata	1	1	within 15 minutes	−
T. infestans	2	1	within 15 minutes	−
T. protracta	10	5	6–60 hours	−
T. protracta	8	4	4–7 days	−

* Interval between infection of insect and collection of feces.

quired on the order of a thousand times more plague bacteria than by subcutaneous injection.

Trypanosoma cruzi is readily transmitted by Triatominae to *Neotoma* spp. and many of these infections are thought to occur by ingestion of infected insects since rats can be readily infected by this route in the laboratory (Ryckman, unpublished data). When Triatominae infected with both *T. cruzi* and *P. pestis* were fed to mice, 4 out of 9 attempts resulted in positive transmission of plague, with only one case being primary pneumonic plague. Although the number

of tests is small, the results suggest an enhancing factor, probably mechanical, and may possibly indicate the potential importance of this mechanism for this vector. Furthermore, the recent findings of Quan and Holdenried (unpublished data) that some *Neotoma* spp. are even more susceptible to plague than the laboratory mouse (Princeton strain) suggests that these animals might readily become infected in nature by ingestion of infected bugs.

Chagas' disease is transmitted to humans by Triatominae when infected fecal material containing *T. cruzi* contacts the conjunctivae, mucous membranes, or an open wound. Seventeen tests were conducted to determine if virulent plague bacilli are passed through the digestive system of the bug into the feces. The only positive feces were from two *Triatoma platensis* whose feces were positive 15 minutes after these two insects completed their meal of infected blood. In the mosquito, Kartman (1953) found as much as 17 per cent of the ingested microfilariae in the dejecta almost immediately after feeding on a filaria-infected dog. It is believed that viable *P. pestis* in Triatominae feces immediately after feeding has heretofore not been observed.

SUMMARY

Eight species in two genera of Triatominae have been tested as vectors of plague. The plague bacilli were found to remain infective in *Triatoma protracta* (Uhler) for 3 days at 30°C., but were negative on the fifth day when the bugs were injected as an aqueous suspension into guinea pigs and mice.

Four adult *Triatoma phyllosoma pallidipennis* (Stål) in 2 different groups transmitted plague by interrupted feeding; i.e., first feeding on an infected mouse and then feeding on a healthy mouse. These transmissions were probably due to contaminated mouth parts. Thirteen similar feedings were negative using 5 other species of *Triatoma*.

Triatoma protracta and *Mestor megistus* (Burmeister) infected with *Trypanosoma cruzi* Chagas and *Pasteurella pestis* (Lehman and Neumann) were fed to mice; 4 positive plague infections resulted one of which was pneumonic; 3 mice were negative. Thirteen other tests with plague-infected Triatominae fed to mice failed to transmit *P. pestis* through the gastrointestinal tract, but produced 3 pneumonic cases.

Triatoma platensis Neiva was the only species to yield plague positive feces out of 17 specimens tested.

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OCCURRENCE OF *HISTOPLASMA CAPSULATUM* AND OTHER HUMAN PATHOGENIC MOLDS IN PANAMANIAN SOIL

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A recent visit to the Republic of Panama provided the opportunity to collect soil specimens for mycologic study. The results of the examination of these samples for the presence of *Histoplasma capsulatum* and other human pathogenic molds are presented in this report.

Over a two-week period, 100 soil samples were collected in several of the more accessible areas of the country. The majority of the specimens were gathered deliberately in areas frequented by chickens, as soil studies carried out previously in Williamson County, Tennessee, in collaboration with Zeidberg (1952, 1953) had demonstrated that a significantly higher percentage of soils gathered from chicken coops and chicken yards yielded *H. capsulatum* than soils obtained from other habitats. The approximate locations of the collecting sites are indicated on the accompanying map (Fig. 1).

All the specimens were scooped directly from the uppermost layer of the soil into 4 oz. collecting bottles, labeled, and flown to Georgia. There the soils were examined for *H. capsulatum* by injecting the supernate from soil suspensions intraperitoneally into mice and culturing small portions of their livers and spleens, and for *Microsporum gypseum* by baiting plates of moistened soil with keratinaceous substrata, viz. pieces of sterilized hair, feathers and cow horn shavings. The details of these procedures are outlined as follows:

A. *H. capsulatum* procedure[1], (Stewart and Meyer, 1932; Emmons, 1949; Ajello and Runyon, 1953):
   1. Approximately 10 ml. of a thoroughly mixed soil sample are suspended in 30 ml. of physiological saline.
   2. The suspension is stirred vigorously and allowed to stand for at least one hour.
   3. Five ml. of the supernatant fluid are pipetted off and one ml. aliquots injected intraperitoneally into each of 4 mice.
   4. The mice are treated once daily for 5 days with 1,000 units of streptomycin and 12,500 units of penicillin to prevent death by any pathogenic bacteria that may be present in the soil.
   5. After 8 weeks the mice are sacrificed and portions of their liver and spleen inoculated into tubes of a neutral dextrose-peptone agar.
   6. The isolation tubes are incubated at 25°C. and examined over a period of 6 weeks for the presence of pathogenic molds.

B. *M. gypseum* procedure (Vanbreuseghem, 1952; Ajello, 1953):
   1. Sterile petri dishes are half filled with soil.

[1] With this method other pathogens such as *Allescheria boydii*, *Coccidioides immitis* and *Cryptococcus neoformans* can be recovered from soil.

TABLE 1

Location and nature of soils yielding pathogenic fungi

Code No. of Positive Soil Samples	Isolate	Nature of Collecting Site	Locality
2	*M. gypseum*	Under tree used as chicken roost	Road between Chorrera and Capira
4	*M. gypseum*	Under wild plum tree used as chicken roost	La Campana
10	*M. gypseum*	Under wild plum tree used as chicken roost	La Campana
11	*H. capsulatum*	Under wild plum tree used as chicken roost	La Campana
14	*M. gypseum*	Soil along fence of chicken yard	Chilibri
15	*M. gypseum*	Soil gathered in front of chicken pen	Chilibri
16	*M. gypseum*	Soil from right side of chicken pen	Chilibri
18	*M. gypseum*	Soil in rear of chicken house	Chilibri
19	*M. gypseum*	Soil around chicken house	Chilibri
20	*M. gypseum*	Soil in rear of chicken house	Chilibri
25	*M. gypseum*	Soil in rear of chicken house	Chilibri
26	*M. gypseum*	Soil in rear of chicken house	Chilibri
27	*M. gypseum*	Soil in rear of chicken house	Chilibri
29	*M. gypseum*	Inside chicken coop	Ciricito
31	*M. gypseum*	Soil under guava tree used as chicken roost	Ciricito
32	*M. gypseum*	Soil under guava tree used as chicken roost	Ciricito Ciricito
37	*M. gypseum*	Under a tree roost	Ciricito
48	*M. gypseum*	Under house	Ciricito
49	*M. gypseum*	Soil in front of house	Ciricito
50	*M. gypseum*	Under house	Ciricito
51	*M. gypseum*	Chicken yard	Ciricito
52	*M. gypseum*	Under yucca tree	Ciricito
53	*M. gypseum*	Under papaya tree	Ciricito
54	*M. gypseum*	Under achiote bush	Ciricito
57	*M. gypseum*	Dry soil under termite study house	Barro Colorado Island
61	*M. gypseum*	Dry soil under termite study house	Barro Colorado Island
62	*M. gypseum*	Dry soil under termite study house	Barro Colorado Island
66	*M. gypseum*	Chicken yard in patio of hotel	Santiago
72	*M. gypseum*	Soil around El Rancho de Coca Cola cock fighting arena	Road between David and La Concepcion
73	*M. gypseum*	Soil around El Rancho de Coca Cola cock fighting arena	Road between David and La Concepcion
76	*M. gypseum*	Cock fighting pit	Road between David and La Concepcion
82	*M. gypseum*	Chicken house	Santa Rosa
83	*A. boydii*	Under nance tree used as chicken roost	Cuipo
85	*M. gypseum*	Under house	Cuipo
88	*M. gypseum*	Under house	Cuipo
89	*M. gypseum*	Under guava tree chicken roost	Cuipo
96	*M. gypseum*	Chicken pen under house	Cuipo
97	*M. gypseum*	Chicken pen under house	Cuipo

2. Sufficient sterile distilled water is added to moisten the soil thoroughly.
3. Short strands of autoclaved hair or pieces of other keratinaceous materials are placed upon the surface of the moist soil.
4. The baited dishes are incubated at room temperature and observed over a period of 8 weeks.
5. Those pieces of bait that become covered with mycelium are examined microscopically and cultured on a medium containing cycloheximide, penicillin and streptomycin. This medium permits the isolation of many human pathogenic fungi from sources heavily contaminated with bacteria and saprophytic molds (Georg, 1953; Ajello and Getz, 1953).

RESULTS

From the 100 soil samples, through use of the mouse technique, one isolate of *H. capsulatum* and one of *A. boydii* were obtained while 36 of the soils, when baited with keratin, yielded the dermatophyte *M. gypseum*. In Table 1 the nature of the collecting sites and geographic location of the positive soils are presented.

Culturally and morphologically, the Panamanian isolate of *H. capsulatum* was basically similar to the human isolates maintained in the culture collection of the Communicable Disease Center's mycology laboratory and to the soil isolates that have been recovered from Williamson and Shelby Counties, Tennessee. The appearance of the La Campana isolate, in a set of primary isolation tubes inoculated with pieces of liver and spleen is seen in Figure 2. It produced tuberculate spores in abundance (Fig. 3) and was readily converted to the yeast-phase by

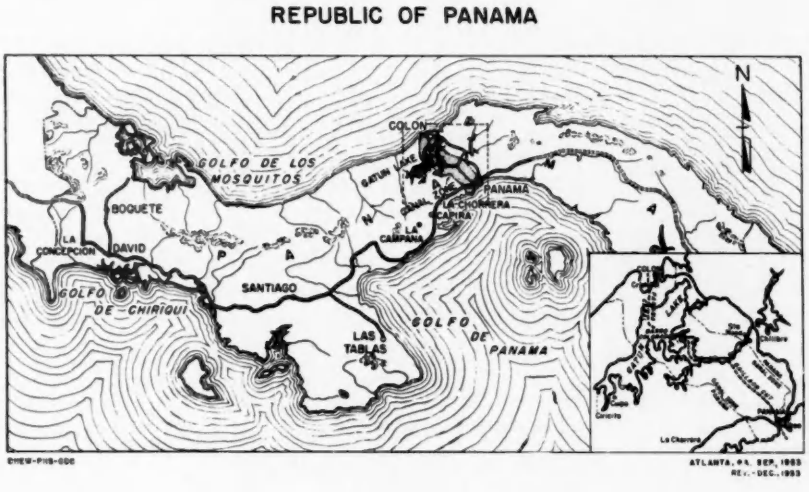

FIG. 1. Map of the Republic of Panama and the Canal Zone indicating soil collecting areas

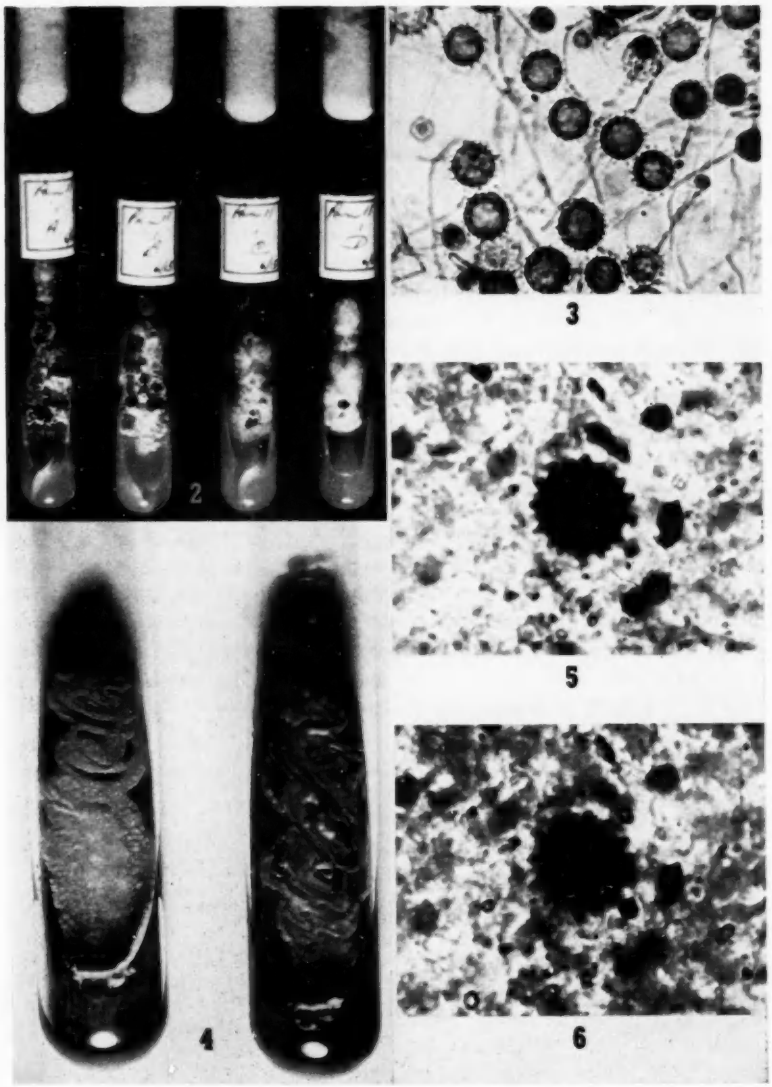

FIG. 2. Appearance of *H. capsulatum* in a group of primary isolation tubes inoculated with pieces of liver (A, B) and spleen (C, D) from mouse No. 1 injected with 1 ml. of soil suspension (Specimen No. 11).

FIG. 3. Tuberculate spores produced by the Panamanian isolate of *H. capsulatum* on Sabouraud dextrose agar. (Original magnification 475×).

FIG. 4. Yeast phase of this isolate obtained by culturing on Francis' glucose cystine blood agar (L) and on Thompson's medium (R) at 37°C.

FIG. 5. Median optical view of a tuberculate spore of *H. capsulatum* filtered from soil specimen No. 11 with a membrane filter (Original magnification 980×).

FIG. 6. Surface view of same spore showing thick wall and tubercles (Original magnification 980×).

FIG. 7. View of site in the village of La Campana, where soil sample that yielded *H. capsulatum* was collected.

subculturing on tubes of Francis' glucose cystine agar (Campbell, 1945) and Thompson's (1945) medium incubated at 37°C. (Fig. 4).

With membrane filters, according to a procedure developed by Gordon and Cupp (1953), it was possible to observe directly the tuberculate spores of *H. capsulatum* that were present in soil specimen No. 11 (Figs. 5, 6).

The demonstration of these spores in soil reconfirms the fact, first established by Emmons (1949), that *H. capsulatum* exists in nature as an actively growing saprophyte since tuberculate spores are never produced *in vivo*[2]. A view of the site in the village of La Campana, where soil sample No. 11 was collected, is presented in Fig. 7.

The isolate of *A. boydii*, a mold capable of causing mycetomas, was in its perfect stage producing both asexual conidia borne on simple conidiophores and ascospores in cleistothecia. Spore suspensions treated with gastric mucin and injected intraperitoneally into mice induced death within 10 days, a finding which had been demonstrated previously with a large series of human and soil isolates of this fungus (Ajello, 1952; Ajello and Anthony, 1953).

Best results in the recovery of *M. gypseum* were obtained with hair. Although in some instances the pieces of feather revealed *M. gypseum* before the other baits, it tended to disappear quickly from the soil plates as the result of the keratinolytic action of various microorganisms. Cow horn shavings were invaded

[2] The observation by Haley (1952) of mycelium and tuberculate spores of *H. capsulatum* in a mouse injected with yeast-phase cells may be accounted for on the basis of the influence of necrotic tissue on diphasic fungi.

by bacteria in a relatively short time and thus they too did not prove to be as satisfactory as hair for isolating *M. gypseum*.

All the isolates of *M. gypseum* sporulated heavily while growing on the baits in the soil plates. Pure cultures were obtained with little difficulty by using the cycloheximide medium previously mentioned.

Subcultures of the Panamanian isolates of *H. capsulatum*, *A. boydii* and three of the *M. gypseums* have been deposited in the American Type Culture Collection, Washington, D. C. and in the Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

DISCUSSION

An anomalous situation has long existed relative to the status of histoplasmosis on the Isthmus of Panama. Despite the fact that the first three known cases of that disease were discovered there by Darling (1906, 1907, 1908) within a period of 8 months (Dec. 7, 1905 to Aug. 6, 1906), only two other cases, one involving a dog (Tomlinson and Grocott, 1945), the other a child (Draheim *et al.*, 1951), have been subsequently encountered in that country.

That histoplasmosis is not a rare disease in Panama has been proven recently by skin-testing surveys carried out by Tucker (1952) and de Pinzón (1952). Their surveys indicate that a benign and not uncommon form of histoplasmosis exists in this region of the world. Of 1,000 individuals entering the Gorgas Hospital in Ancon, the Canal Zone, 39 per cent were found by Tucker to give positive skin test reactions to histoplasmin; while sensitivity studies carried out in the Gatun Lake villages of Cuipo and Ciricito by de Pinzón revealed that 25 per cent were reactors.

Despite this immunologic evidence of the existence of a prevalent but benign form of histoplasmosis and records of 5 fatal cases of that disease, the etiologic agent, *H. capsulatum*, up to the present instance, has never been isolated from any source in Panama. The recovery of *H. capsulatum* from a soil specimen gathered in the village of La Campana, not only represents the first isolate of that organism ever obtained in Panama, but also unequivocally establishes this parasite as being indigenous to this area, thus dispelling any doubts relative to the endemicity of histoplasmosis in Panama.

The recovery of *A. boydii* from soil gathered in the village of Cuipo confirms the fact previously established by Emmons (1952) and Ajello (1952) that this mold exists in nature as a saprophyte. It is interesting to note that as yet no instances of human infection by *A. boydii* have been recorded in Panama. Undoubtedly this is a reflection of the basically saprophytic nature of *A. boydii* and perhaps, to a lesser extent, the general lack of available medical mycological diagnostic services in many parts of the Isthmus.

Despite the demonstrated prevalence of *M. gypseum* in soil throughout the Republic, few instances of infection by that parasite have been observed in Panama. Shrager (1952) has noted the only two known Panamanian cases of ringworm caused by this interesting organism. These were found among several hundred cases of ringworm examined at the Gorgas Hospital.

The percentage of recoveries of *M. gypseum* from Panamanian soils (36%) is in close agreement with that obtained from soils of Tennessee (35.1%) and Georgia (26.2%) (Ajello, 1953). A small number of specimens gathered in the Province of Ontario, Canada[2] and W. Virginia[3] were found to be positive for *M. gypseum*, 16.6 per cent and 12.5 per cent, respectively. All these findings suggest that this dermatophyte is remarkably abundant and widespread in soil.

The Panamanian findings are in no sense unique, since soils gathered in many areas of the United States and Canada by several investigators have yielded a remarkable variety of human pathogenic fungi (Stewart and Meyer, 1922; Emmons, 1949, 1951). It is to be expected that when similar studies are carried out elsewhere in the world a similar pattern will unfold.

ACKNOWLEDGMENTS

These studies could not have been made without the deep interest and cooperation of Dr. Herbert C. Clark, Director of the Gorgas Memorial Laboratory, Panama, R. of Panama; Dr. Carl Johnson of the Board of Health Laboratory, Gorgas Hospital, Ancon, Canal Zone; and Senora Teresina P. de Pinzón.

To Dr. Harold Trapido of the Gorgas Memorial Laboratory, who was our kind and generous host throughout the stay in Panama, I wish to express sincere gratitude.

Credit is due to Dr. L. C. Runyon for technical assistance in carrying out the mouse isolation procedures and to my wife, Gloria Ajello, for recording all pertinent field data.

SUMMARY

Soil studies carried out with specimens collected on the Isthmus of Panama yielded isolates of *Histoplasma capsulatum*, *Allescheria boydii* and *Microsporum gypseum*. This constitutes the first record of the occurrence of these three human pathogenic molds in the soil of tropical America. The techniques used in the recovery of these fungi are outlined, and the implication of the findings are discussed.

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DIAGNOSIS OF SCHISTOSOMIASIS BY COMPLEMENT-FIXATION

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Interest in serologic methods for the diagnosis of schistosomiasis has been stimulated by the inability at times to demonstrate eggs of the parasite in excreta from infected individuals. The problem of the occult infection assumes special importance in areas where schistosomiasis is endemic and infections tend to be chronic; under these conditions a definitive diagnosis becomes possible, in many instances, only after repeated examination of stools or urine. The feasibility of a serologic solution to the problem was indicated early by Yoshimoto (1910), who demonstrated complement-fixing antibody in sera from five individuals with *Schistosoma japonicum* infection; the antigen used was an alcoholic extract of adult worms from an infected calf. Subsequently Fairley (1919) derived antigen from the livers of cercaria-infected snails, and showed that the specific reactive components could be extracted in salt solution as well as in alcohol. Saline and alcoholic extracts of adult *S. japonicum* were later prepared and used in comparative tests by Miyaji and Imai (1928). An essential deficiency of many antigens, however, was their tendency to react with syphilitic serum; this was a characteristic particularly of the alcoholic extracts prepared from infected snail livers. Extracts in salt solution exhibited fewer nonspecific reactions, and were considered in general the antigens of choice.

The present studies originated in attempts to develop an improved complement-fixing antigen from adult schistosomes. The method applied in removing nonspecific constituents of the worms was suggested by the experience of Taliaferro *et al.* (1928), who had observed that substances responsible for the false precipitin reactions with syphilitic serum could be extracted from cercarial concentrates with organic solvents. Following certain essential innovations such as the use of cold anhydrous ether for preliminary extraction, the method proved effective also in removing undesirable components from the adult schistosome. Specifically reactive components of the worm residue were readily extracted in buffered salt solution. The improved antigen was evaluated in a quantitatively standardized complement-fixation procedure. Details of the methods, together with results obtained in comparative tests with schistosome-infected, healthy, and syphilitic individuals are presented herein. A separate report presents collateral studies (Horstman *et al.*, 1954) in which the complement-fixation test was compared with an intradermal test using the same purified antigen.

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MATERIALS AND METHODS

Infection of animals and harvest of adult schistosomes. The adult worms used in preparing antigen were obtained from the liver and from the mesenteric and portal veins of experimentally infected hamsters. Just prior to exposure to infective cercariae, the animals were placed for 10 minutes in a glass cylinder with warm tap water to induce urination and defecation. Those to be infected with *S. mansoni* were then immersed partially for 30–40 minutes in a second cylinder containing cercariae in previously aerated tap water. Hamsters to be infected with *S. japonicum* were first shaved on the lower half of the back and abdomen and inoculated intraperitoneally with viable cercariae; they were then exposed to additional cercariae by the partial immersion technic, as with *S. mansoni.* The animals were sacrificed when viable parasite eggs appeared in the fecal pellets, usually 6–8 weeks after infection, and the adult schistosomes were recovered. The worms were washed several times with sterile 0.85 per cent NaCl solution and once with distilled water, then transferred for desiccation to 10 ml. ampoules. The contents were quickly frozen in a mixture of solid carbon dioxide and ethanol (−78°C.), and dehydrated under reduced pressure from the frozen state. After 16–18 hours dehydration the ampoules were sealed hermetically under vacuum using a cross-fire torch, and refrigerated with solid carbon dioxide until material sufficient for an antigen extraction was accumulated. Additional animals were exposed at regular intervals so that the period between worm harvests was usually 3–4 weeks.

Antigen extraction. The antigen finally adopted was a buffered saline[4] extract of ether-extracted schistosomes. The use of anhydrous ether[5] for preliminary extraction was found to be essential; pre-extraction with anesthesia ether, anhydrous ethanol, or benzene had deleterious effects on the antigen. A glass tissue-grinder powered with a variable speed electric motor, was used to facilitate extraction. Prior to an ether extraction, both grinder and ether were chilled to −20°C. in a refrigerator. A 100 mgm. quantity of the desiccated schistosomes was then transferred quantitatively to the chilled grinder, 10 ml. of cold anhydrous ether were added, and the mixture ground for 10 minutes with the grinder cylinder immersed in a calcium chloride ice-water bath (−15 to −18°C.); the motor was run at low speed to minimize evolution of heat in the grinder. The ethereal suspension of ground worms was poured into a chilled glass centrifuge tube and placed temporarily in a refrigerator (3–6°C.). Five ml. of the cold ether were next added to the small amount of worm residue in the grinder and grinding resumed for 2 minutes. This second suspension was then combined with

[4] The stock solution consisted of 83.8 gm. NaCl, 2.52 gm. $NaHCO_3$, 3.00 gm. sodium 5,5-diethyl barbiturate, and 4.60 gm. 5,5-diethyl barbituric acid in distilled water sufficient to make 2000 ml. (Mayer *et al.*, 1948). The acid was dissolved in 500 ml. hot distilled water and added to the solution of the other reagents. The whole was then cooled to room temperature, and made up finally to 2000 ml. with distilled water. Before use, one volume of the stock solution was diluted by adding four volumes of distilled water; the pH of the diluted solution was 7.3–7.4.

[5] Mallinckrodt Chemical Works, New York, Philadelphia, St. Louis.

the first, and the whole spun for 30 minutes at 850 G. in a refrigerated (3–6°C.) angle centrifuge. Following centrifugation the supernatant ether was immediately decanted and traces of ether vapor aspirated from the centrifuge tube and grinder through a Richard's filter pump. For the final saline extraction the ether-extracted worm residue was returned quantitatively to the grinder using 10 ml. of veronal bicarbonate buffered salt solution (VBS),[4] and the mixture ground slowly for 10 minutes at room temperature. The resulting suspension was poured into a cellulose nitrate centrifuge tube. The small amount of worm residue remaining in the grinder was then suspended by grinding for 2 minutes with an additional 5 ml. of VBS, and combined with the bulk of suspension. Finally the pooled material was transferred to a refrigerator (3–6°C.), where extraction was allowed to proceed for 4 hours. The suspension was then spun for 30 minutes at 850 G. in the refrigerated (3–6°C.) centrifuge and the opalescent supernate removed carefully to a beaker. One ml. volumes of the extract were dispensed in 5 ml. glass ampoules; the contents were shell-frozen in solid carbon dioxide and ethanol (−78°C.), and dehydrated under reduced pressure from the frozen state.[6] After 16–18 hours dehydration the ampoules were sealed hermetically under vacuum, and stored at −20°C.; when required for tests the contents of an ampoule were rehydrated with 1 ml. of distilled water.

For comparative tests saline extracts were prepared concurrently from worms of the same lot not subjected to preliminary extraction with ether; 100 mgm. quantities of the desiccated schistosomes were extracted with VBS using the procedure given for ether-extracted worms.

Sera. Sera representing *S. mansoni* infections were obtained from 98 native Puerto Ricans, of whom 66 were males aged 3 to 60 years, and 32 were females, 12 to 32 years of age. The specific diagnosis in all but three instances had been established by demonstration of the parasite eggs in stools; in two of the exceptional cases the eggs were discovered by rectal biopsy, and in the third by examination of a surgically-removed gall bladder. The sera were preserved in tubes containing dried merthiolate (sodium ethyl mercuri thiosalicylate)[7] sufficient to give a final concentration of about 0.5 mgm. per ml., and were shipped by air mail to the Army Medical Service Graduate School. The six sera from *S. japonicum* infections were contributed by U. S. Army personnel infected in the Philippine Islands during World War II; the diagnosis had been established in each instance by demonstration of the parasite eggs in stools. Normal sera were obtained from healthy donors among the laboratory staff, and from patients at the Walter Reed Army Hospital who had never resided in an area where schistosomiasis is encountered; all were negative in serologic tests for syphilis. Syphilitic sera were procured from 28 individuals admitted for chemotherapy to the Polk Health Center, Washington, D. C.; none had been exposed to schistosomiasis,

[6] Where facilities for desiccation from the frozen state are not available, 1.0 ml. volumes of the extract may be sealed in small test tubes with paraffin-coated corks, and preserved in the frozen state (−20° C). In a limited series of comparative tests extract so stored remained unaltered in antigenic activity for at least two months.

[7] Eli Lilly and Company, Indianapolis, Indiana.

and all reacted strongly in serologic tests for syphilis. The sera were stored at −20°C.; just prior to testing the required volume of thawed serum was heated for 30 minutes in the water-bath at 56°C.

Complement-fixation procedure. The standard diluent was a veronal bicarbonate buffered salt solution (VBS).[4] Preserved sheep's blood (Bukantz *et al.*, 1946) served as source of erythrocytes; glycerolized hemolytic antiserum and desiccated guinea pig complement (C′) were procured from commercial firms. The reagents were standardized quantitatively as required for a sensitive diagnostic complement-fixation procedure (Kent, 1950). Suspensions of washed sheep's erythrocytes were adjusted spectrophotometrically to contain 5×10^8 cells per ml., and sensitized with an optimal concentration of hemolysin. The fifty per cent hemolytic unit (C'_{H50}) of complement was determined by a precise spectrophotometric technic. The standard volume for all procedures was 1.5 ml., aliquot 0.3 ml. volumes being allotted the five essential reagents in the complement-fixation test; adjustments to standard volume were made when necessary with VBS. In the antigen titrations and diagnostic tests, 0.1 ml. of undiluted or diluted serum plus 0.2 ml. of VBS was incubated 16–18 hours at 3–6°C. with 0.3 ml. each of complement (3 C'_{H50}) and antigen. Sensitized erythrocytes (0.6 ml.) were then added, and 30 minutes in the water-bath at 37°C. allowed for hemolysis. Tests were read by visual comparison with standards simulating the possible findings; i.e., reaction (4+, 3+, 2+), weak reaction (1+, ±), and no reaction (−). A detailed account of the procedures has been presented in connection with comprehensive studies of sporotrichosis (Nordén, 1951).

The results obtained in a representative antigen titration are given in Table 1. It should be noted that the antibody-containing serum was diluted in non-reactive human serum (Kent, 1952), the antigen in VBS. A 1:20 dilution of antigen, in this instance, was selected for use in diagnostic tests; there was no evidence of anticomplementary activity in antigen controls.

TABLE 1

Standardization of a saline extract of ether-extracted adult S. mansoni *for use as antigen in the complement-fixation test*

ANTIGEN DILUTED IN SALT SOLUTION 1:	RESULTS* WITH S. MANSONI SERUM DILUTED IN NORMAL HUMAN SERUM 1:							ANTIGEN CONTROLS
	1	2	4	8	16	32	64	
5	4	4	4	4	4	3	−	−
10	4	4	4	4	4	3	−	−
20†	4	4	4	4	4	3	−	−
40	4	4	4	4	3	1	−	−
Serum controls	−	−	−	−	−	−	−	

* The recorded numerical values represent degree of complement-fixation, the negative sign (−), no fixation.

† The dilution of antigen selected for diagnostic tests.

RESULTS

Sera from 66 male and 32 female Puerto Rican natives, all known to have been infected with *S. mansoni*, were tested for complement-fixing antibody using antigens prepared by saline extraction from untreated (A) and ether-extracted (B) adult *S. mansoni*. Preliminary examination of the findings revealed no basis

TABLE 2

Relative sensitivity and specificity for schistosomiasis of antigens prepared by saline extraction of untreated (A) and ether-extracted (B) adult S. mansoni

INDIVIDUALS TESTED		S. MANSONI ANTIGEN	INDIVIDUALS EXHIBITING THE GIVEN SEROLOGIC RESULT*		
Diagnostic status	Number		Reaction	Weak reaction	No reaction
S. mansoni infection	98	A	96	0	2
		B	95	1	2
Healthy	46	A	0	0	46
		B	0	0	46
Syphilitic†	28	A	9	2	17
		B	0	0	28

* Classified as reaction (4+, 3+, 2+), weak reaction (1+, ±) or no reaction (−).
† All strongly reactive in serologic tests for syphilis.

TABLE 3

Reactivity of S. mansoni *and* S. japonicum *sera with antigens from the homologous and heterologous adult schistosomes*

DIAGNOSTIC STATUS	SERUM NO.	S. MANSONI ANTIGEN*					S. JAPONICUM ANTIGEN*				
		Results† with serum diluted in salt solution 1:									
		1	2	4	8	16	1	2	4	8	16
S. mansoni infection	43	4	3	1	±	−	4	3	2	1	−
	59	4	4	3	1	±	4	4	4	2	1
	75	4	3	2	1	±	4	4	3	1	±
	76	4	3	1	±	−	4	3	2	1	±
	97	4	2	1	−	−	4	3	2	±	−
	113	4	3	2	1	−	4	4	3	1	±
S. japonicum infection‡	1	1					−				
	2	2					3				
	3	±					1				
	4	2					2				
	5	1					±				
	6	−					±				

* Saline extracts of the ether-extracted schistosomes.
† The recorded numerical values represent degree of complement-fixation, the negative sign (−), no fixation.
‡ The *S. japonicum* sera were not tested in serial dilution.

for a differentiation based upon sex of the donor. The results of the comparative tests are summarized in Table 2. Of the 98 persons tested, 96 reacted with the antigen (A) prepared from untreated schistosomes. Ninety-five of this group reacted, and one showed a weak reaction, with antigen (B) prepared from ether-extracted worms. The antigens thus appeared all but identical in sensitivity.

Groups of healthy and syphilitic individuals were tested similarly to determine the relative frequency of nonspecific reactions with the two antigens. The findings appear also in Table 2; as already indicated herein, the syphilitic donors reacted strongly in serologic tests for syphilis. All 46 healthy persons were negative in tests with both schistosomal antigens. In the group of 28 syphilitic donors, however, 9 reacted and 2 showed weak reactions in the test with antigen (A) derived from untreated schistosomes; none reacted in the test with antigen (B) from ether-extracted worms. The technic of preliminary extraction with ether was followed in all subsequent preparations of antigen.

To obtain some estimate of the species specificity of the serologic reaction, sera from 6 patients with *S. mansoni* and 6 with *S. japonicum* infections were tested with antigens prepared from the homologous and heterologous species of worm. The results are given in detail in Table 3; the *S. japonicum* sera, being of low antibody content, were not examined in serial dilution. Sera from the two closely-related infections could not be differentiated on the basis of their reactivity with antigens from the homologous and heterologous parasites.

DISCUSSION

It is not surprising that substances reactive with syphilitic serum should occur as normal tissue components of the adult schistosome. As Weil (1941) pointed out in his excellent review of the Wassermann and related antigens, ". . . immunological investigation has revealed the rather ubiquitous presence of such substances in living matter." Being essentially lipid in nature these materials exhibit some degree of solubility in organic solvents, and this characteristic may be used to advantage in effecting their separation from specifically-reactive components in an antigen complex. This was the approach followed by Taliaferro *et al.* (1928) in preparing schistosomal antigen for a precipitin test. Dried cercarial concentrates from infected snail livers were subjected to Soxhlet extraction with ether and/or anhydrous ethanol prior to final extraction with Coca's solution; the resulting antigens showed a marked reduction in the tendency to react with syphilitic serum. For the present studies attention was directed to the adult schistosome principally because of its mass and the relative ease with which it could be freed of host tissue contaminants. The method of choice for preliminary extraction proved to be brief treatment in the cold with anhydrous ether. By this procedure worm components responsible for the cross reaction with syphilitic serum were removed without appreciably altering the specific reactive capacity of the antigen (Table 2). It is worth reiterating in this connection that other lipid solvents such as anesthesia ether, anhydrous ethanol, and benzene were less satisfactory for preliminary extraction, the final antigens not infrequently showing reduced activity or undesirable anticomplementary properties. The antigens

used in the present studies were regularly free of anticomplementary activity (Table 1). This experience is in some contrast to that of Wright *et al.* (1947), who found saline extracts of adult schistosomes so anticomplementary as to warrant their rejection for serologic tests. Some explanation for the success of the current method may lie in immediate preservation of the worms by desiccation from the frozen state, and extraction of the dried tissue under conditions of time and temperature less likely to be associated with degenerative changes.

Tests with the improved antigen (Table 2) provide some index of the frequency with which complement-fixing antibody may be encountered in areas where schistosomiasis is endemic. Ninety-six of 98 persons known to have been infected with *S. mansoni* reacted in the serologic tests; two were negative. The latter may well have been cases of relatively recent infection. While both were excreting eggs of the parasite, the possibility exists that circulatory antibody had not yet reached a detectable level. It was apparent from the findings that the complement-fixation test might provide valuable assistance in diagnosis; an advantage of the test would be that circulatory antibody is continuously demonstrable once it attains threshold levels, whereas eggs of the parasite may be detected only intermittently by examination of excreta. The majority (81) of the group had received treatment for their infections, and as a result many had been free of parasite eggs for some time prior to serologic examination. Of special interest are seven of this group who had been stool-negative for 4–6 years, and 5 who had been negative for 1–3 years; all reacted in the complement-fixation test for schistosomiasis. In the small group (17) of untreated individuals, similarly, there were stool negative individuals whose infections had been discovered, two by rectal biopsy and a third by examination of a surgically-removed gall bladder; these likewise reacted in the serologic test. There was ample evidence therefore for the persistence of circulatory antibody in persons without overt evidence of infection, and it appeared that the complement-fixation test might provide a better index of the number of infected persons than is afforded by methods which rely on direct demonstration of the parasite. Evidence supporting this conclusion was obtained during a subsequent survey of Puerto Rican soliders (Horstman *et al.*, 1954) in which the complement-fixation test and an intradermal test using the new purified antigen were employed in conjunction with standard methods of stool examination.

While the specificity of the test for schistosomiasis has been markedly improved, the possibility remains that an exceptional individual with treponematosis may react even with ether-extracted schistosomal antigen. In serodiagnostic practice accordingly an attempt should be made to identify the person with syphilis or other treponemal infection. At this laboratory all sera submitted for the diagnosis of parasitic disease are tested concurrently with a cardiolipin antigen,[8] and the result is taken into consideration in final evaluation of the find-

[8] This antigen was an ethanol solution containing cardiolipin 0.0175 per cent, purified lecithin 0.0875 per cent, and cholesterol 0.30 per cent, as specified by Maltaner and Maltaner (1945); a 1:250 dilution in VBS has been shown (Kent, 1950) to be optimal for use under the conditions of the complement-fixation test, as described herein.

ings. It is essential also to consider the possibility of "false" serologic reactions due to related helminthic or other infections. During the course of these studies, a patient with *Trichinella spiralis* infection was tested at intervals of 4, 7, and 10 weeks following establishment of the diagnosis by muscle biopsy. The 4 and 7 week sera showed weak reactions, and the 10 week serum was negative in the test for schistosomiasis; it is of interest that all three sera reacted in a concurrent test with unpurified schistosomal antigen. Sera from four cases of hookworm infection were examined similarly on another occasion; they were negative in tests with unpurified as well as ether-extracted antigen.

SUMMARY

An improved antigen for the complement-fixation test has been prepared by extracting desiccated adult schistosomes with anhydrous ether in the cold (−15 to −18°C.) prior to final extraction with buffered salt solution. Preliminary extraction with ether markedly reduced a tendency of the antigen to react with syphilitic serum without appreciably altering its capacity to fix complement specifically with serum from schistosome-infected individuals.

Sera from persons infected with *S. mansoni* or *S. japonicum* could not be differentiated on the basis of their capacity to react with antigens from the homologous and heterologous parasites.

The complement-fixation test is discussed from the point of view of its value in the diagnosis of schistosomiasis.

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SCHISTOSOMIASIS MANSONI IN PUERTO RICAN SOLDIERS

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Schistosomiasis mansoni has been known to be endemic in Puerto Rico since 1904, when the eggs of this parasite were first found by Dr. Gonzalez Martinez. A number of studies have since been carried out to determine the prevalence of the disease. Hoffman and Faust (1934) made extensive studies of the epidemiology and geographical distribution on the Island and found the disease to be widely but irregularly distributed. Dammin and Weller (1945–6) reported an incidence of 9.97 per cent among 19,339 men on whom a single stool examination was done. Rodriguez-Molina and Schwachman (1947) reported that 14.6 per cent of a group of 15,831 Puerto Rican men between 18 and 38 years of age were positive when a single stool examination was done by a "special concentration technique".

The incidence of the disease is generally believed to be higher than these figures indicate because of the frequency with which it is encountered as an incidental finding. Also, at least one autopsy study seemed to indicate that about 40 per cent of the men in this highly endemic area are infected with this parasite (Pons *et al.*, 1950). The present study was undertaken to try to determine the incidence of infection with *S. mansoni* among asymptomatic Puerto Rican troops and to compare several methods of diagnosis as screening tests.

MATERIALS AND METHODS

This survey was carried out under field conditions during maneuvers and included 276 Puerto Rican soldiers. They were all members of the regular army and represented a geographical cross section of the Island. They had undergone recent physical examinations and had been found physically qualified for military duty. All denied symptoms of gastrointestinal disturbance or other symptoms suggestive of schistosomiasis. The following studies were carried out on each soldier:

(a) A medical history was taken concerning any previous diagnosis of schistosomiasis or treatment for this condition.

(b) A stool examination.

(c) A complement-fixation test.

(d) An intradermal test.

The stools were examined by the direct smear and the AMS III concentration technics (Hunter *et al.*, 1948). Those specimens found negative were repeated using the AMS III technic following alcohol sedimentation (Jahnes *et al.*, 1947).

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The complement-fixation tests were performed using as antigen a whole-worm extract described and evaluated by Chaffee, Bauman and Shapilo. They found this to be highly specific. The intradermal test was done utilizing a 1:10,000 dilution of the adult worm extract used in the complement fixation studies to which phenol as a preservative had been added (Jahnes *et al.*, 1947). Approximately 2/100 of a cc. of the diluted antigen was injected intradermally on the inner aspect of the right mid-forearm and a similar amount of buffered saline and phenol was injected in the left forearm as control. Reactions were read in 20 minutes and only those which were significantly different from the control in both wheal and flare were considered positive. This test gave no false positive reactions in 158 control individuals who had never been exposed to schistosomiasis.

RESULTS

The studies showed that 18.8 per cent of the 276 soldiers studied had positive stools for *S. mansoni* by the concentration technics described. The complement-fixation test was positive in 43.5 per cent of the group and the intradermal test was positive in 45.0 per cent.

The results, broken down to demonstrate the correlation of the intradermal and complement-fixation test to the stool studies, are shown in Table 1. There were 53 soldiers with positive stools and 49 or 92.5 per cent had both positive intradermal and complement-fixation tests. The group of 223 soldiers with negative stools showed both the intradermal and the complement-fixation test to be positive in 25.1 per cent and both of these tests to be negative in 57.0 per cent. The remaining 17.9 per cent of the negative stool group showed a positive intradermal test with a negative complement-fixation test in 11.2 per cent and a positive complement-fixation test with a negative intradermal test in 6.7 per cent.

Seven, or 2.5 per cent, of the 276 gave a positive history of a diagnosis of schistosomiasis in the past. The diagnosis had been made in all seven patients by previous stool examination. Five of these had received treatment with fuadin, but the dosage was unknown. Of these five, four were positive on stool examination and also gave positive results to complement-fixation and intradermal tests. The other patient reacted positively to the intradermal test but was negative to complement-fixation and stool examination. Both of the untreated patients

TABLE 1

Results of studies for schistosomiasis in 276 Puerto Rican soldiers

LABORATORY FINDINGS	STOOL POSITIVE (53)		STOOL NEGATIVE (223)	
	C-F* Pos	C-F Neg	C-F Pos	C-F Neg
ID† Pos	49 (92.5%)	1 (1.9%)	56 (25.1%)	25 (11.2%)
ID Neg	0	3 (5.7%)	15 (6.7%)	27 (57.0%)

* Complement-fixation test

† Intradermal test

gave positive complement-fixation and intradermal tests and one had a positive stool.

DISCUSSION

The results of these studies show a close correlation in the positive stool group. The positive results obtained by the intradermal and complement-fixation tests in the negative stool group should not be considered as false positive reactions. The diagnosis by stool examination is limited because of the sporadic and intermittent nature of the discharge of the eggs in the stools, the variable prepatent period of the disease, and the resistance to egg penetration in chronic schistosomiasis where the tissues are fibrosed. Furthermore, control studies have shown few false positive reactions.

It seems possible that the poorer correlation in the negative stool group results from the fact that these persons have smaller infections and, as a result, less antibody response. The incidence of false negative reactions using this antigen for skin tests in individuals with positive stools or rectal biopsies was found by Bauman *et al.* (unpublished) to be very low. The incidence of 43 per cent for positive complement-fixation and 45 per cent positive intradermal tests does not seem excessive in a group of young men in this highly endemic area. The high incidence of schistosomiasis among asymptomatic persons is also not uncommon and has been described frequently in Puerto Rico (Kehoe and Long, 1951; Faust, 1949).

The technic for the complement-fixation and intradermal tests as applied to the study of schistosomiasis is not new. The use of these methods utilizing both adult worm and cercarial antigens has been reviewed by a number of authors (Oliver Gonzalez *et al.*, 1944; Bozicevich *et al.*, 1947). Mayer and Pifano (1949), using an adult worm antigen, have found a high degree of specificity.

Persons with a positive skin or complement fixation test do not necesasrily have an active infection nor do they need treatment or constitute a public health problem. These tests will only detect antibodies and may remain positive for many years after the infection has been cleared. They do, however, indicate the need for further study using multiple stool examinations or rectal biopsy to detect the presence of eggs in the stool or mucosa. The advantages of early diagnosis and treatment of this disease are obvious.

SUMMARY AND CONCLUSIONS

The results of a study employing various methods of detecting schistosomiasis in 276 asymptomatic Puerto Rican soldiers are reported. Forty-five per cent showed a positive intradermal test, 43.5 per cent showed a positive complement-fixation test and 18.8 per cent had positive stools for ova. The intradermal and complement-fixation tests appear to be good screening tests and are simple to perform.

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USE OF A HEXYLRESORCINOL TABLET IN THE ENEMA TREATMENT OF WHIPWORM INFECTION

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The whipworm (*Trichocephalus trichiurus*) has been shown to be an important agent of disease in some areas (Fernan-Nunez, 1927; Pallister, 1933; Plessen, 1945; Getz, 1945; Whittier *et al.*, 1945; Einhorn and Miller, 1946; Jung and Beaver, 1951). In southeastern Louisiana it is one of the most frequent causes of chronic dysentery in young children. While oral medication for this condition has been found to be generally ineffective, the efficacy of the 0.2 per cent hexylresorcinol retention enema in reducing the infection to subclinical level has been established (Jung and Beaver, 1951; Basnuevo *et al.*, 1952).

Heretofore in the United States it has been necessary to make up a suspension from pure powdered hexylresorcinol. The inconvenient operation of weighing can be obviated by use of a tablet containing 0.4 grams of hexylresorcinol.[1] Four hundred cubic centimeters of hexylresorcinol suspension can be readily prepared by dropping two of the tablets in that amount of distilled water and shaking vigorously.

Before instilling hexylresorcinol suspension the bowel is cleansed with several normal saline enemas. The buttocks, thighs and perineum are then covered with a protective coating of petroleum jelly, since the spilled suspension may cause second degree burns. The enema is instilled slowly with the objective of filling the large intestine to the cecum. This requires up to 500 cc. in the average pediatric patient (2–4 years old). The patient is encouraged to retain the enema for thirty minutes. If necessary, this is done by taping or holding the buttocks together. After an hour if the treatment enema is not returned spontaneously, an additional small tap-water enema is given to stimulate return. Proctoscopy of patients immediately after treatment and on several successive days thereafter has failed to show any damage to the mucosa. That the skin is burned by this concentration of hexylresorcinol and not the mucosa is explained by the protection afforded the mucosa by the film of mucus covering it.

Twenty-two patients with whipworm infection have been treated by use of hexylresorcinol enemas prepared from tablets.[2] Stool examinations were done before and after treatment, and when possible, proctoscopy and egg-counts by the direct smear method (Beaver, 1950) were done as well.

In six patients who had light, nondysenteric infections before treatment the relationship of symptoms to the whipworm infection was doubtful and in all cases the basic illness proved to be other than trichocephaliasis. Information relating to these patients is presented in Table 1. Although the worm burden

[1] The tablets of hexylresorcinol used in this study were supplied by Sharp and Dohme.

[2] On the Pediatric Services of the Charity Hospital of Louisiana at New Orleans.

TABLE 1

Presenting signs and symptoms and egg counts in six patients with light whipworm infection, treated with hexylresorcinol enema

Patient Number	Race, Sex, Age*	Pre-treatment		Post-treatment	
		Eggs/mgm. feces	Signs, symptoms	Eggs/mgm. feces	Signs, symptoms
1	CM4	7–9	Abdominal pain	3	Same
2	CM2	25	Hepatomegaly, eosinophilia, fever; proctoscopy negative	3	Same
3	CM2½	20	Abdominal pain, diarrhea		Asymptomatic
4	WM10	10	Fever, vomiting Ascaris	12	Asymptomatic
5	CF2	10	Abdominal pain, vomiting Ascaris	1	Asymptomatic
6	CM10	6	Abdominal pains, proctoscopy negative	6	Same

* C = Colored; W = White; M = Male; F = Female. Numbers (Column 2) refer to age in years.

was lowered in most cases by treatment, clinical improvement, if any, was not ascribed to this but to therapy directed against the basic disease.

Sixteen patients had heavy, dysenteric infections with worms seen attached to the mucosa of the rectum during prolapse or proctoscopy. The stools of all these patients contained more than 100 *Trichocephalus* eggs per milligram of feces. The information relating to the effects of treatment in these heavily infected patients is given below in Table 2. In the twelve instances in which egg-counts were done after treatment there was a diminution of the egg-count of from 80 to over 99 per cent. In one case the infection was apparently eradicated. In four of five cases in which proctoscopy was repeated after treatment the worms had been eradicated from the portion of the bowel visualized. In all five individuals proctoscoped after treatment there was a striking improvement in the appearance of the bowel. In all of the heavily infected patients there was marked symptomatic improvement within two days after treatment.

Two patients required two hexylresorcinol enemas for the above changes to be accomplished. Post-treatment egg-counts tabulated in these cases refer to the counts after the second treatment. All other patients received but one enema.

In five of the heavily infected patients there was simultaneous infection with *Endamoeba histolytica*. The predisposition of patients with heavy whipworm infection to amebiasis has been previously pointed out (Jung and Beaver, 1951). These five patients received treatment for their amebiasis only after improvement had been obtained with the hexylresorcinol enema alone.

The retention enema prepared from hexylresorcinol tablets provided about as good results in this series of patients as those reported previously with enemas prepared from the powder. The tablet offers the advantage of convenience of

TABLE 2

Presenting signs and symptoms and egg counts in sixteen patients with heavy whipworm infection, treated with hexylresorcinol enema

Patient Number	Race, Sex, Age†	Pre-treatment			Post-treatment	
		Eggs/mgm. feces	Proctoscopy	Symptoms	Eggs/mgm. feces	Proctoscopy
7	CF2	201		Diarrhea	1	
8	WM4	>300	+	Dysentery, rectal prolapse	10	−
9	CM2	400	+	Dysentery	10	−
10	CF3	109	+	Dysentery, rectal prolapse	1	
11	WF5	>300	+	Dysentery	0	
12	CF4	250		Dysentery	10	
13	CM4½	150	+	Dysentery	28	
14	CM3	>300	+	Dysentery		−
15	WM2	150	+	Dysentery	2	
16	WF2½	>300	+	Dysentery		
17	CF2	150	+	Dysentery		
18	CM2		+	Dysentery, rectal prolapse		
19	CM2	>300	+	Dysentery	6	−
20	CM3	>300	+	Dysentery	1	+*
21	CF2	>300	+	Dysentery	15	
22	CF2	>300	+	Dysentery	12	

+ indicates friable hyperemic mucosa with worms attached seen at proctoscopy; − indicates normal.

* Proctoscopy after first treatment but not after second.

† Abbreviations as in Table 1.

preparation. Experiences with oral medication have been so disappointing that the hexylresorcinol enema may be designated the treatment of choice in heavy whipworm infection.

SUMMARY

Sixteen patients with heavy whipworm infection were improved in three respects after treatment with a hexylresorcinol retention enema prepared from tablets of hexylresorcinol. There was a great reduction in the egg-count; symptoms of diarrhea or dysentery were relieved; worms were eradicated from that portion of the bowel which could be visualized on proctoscopy. Treatment with hexylresorcinol enema did not obviously benefit six patients with light infections. In only one case was the infection eradicated. It is concluded that the hexylresorcinol enema made from tablets is about as effective as that prepared from the powder and is more conveniently prepared.

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DDT RESISTANCE IN EGYPTIAN BODY LICE*

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Resistance of the body louse (*Pediculus humanus corporis* Deg.) to DDT was first observed in Korea in 1950–51 (Hurlbut *et al*, 1952). DDT had been used extensively during a typhus epidemic in Japan and Korea in the winter of 1945–46 with apparent success (Scoville, 1948). At that time over 2 million persons were treated with DDT powder and occupants of jails, hospitals, vagrant shelters and repatriation camps were routinely dusted. Although not so extensively used during the intervening years until 1950, DDT was presumably available at least to personnel associated with the occupation forces. With the outbreak of the Korean War it was again used routinely on a large scale. However, in the late winter of 1950–51 it became evident that a degree of resistance existed which rendered DDT no longer effective as a delousing agent. Upon further investigation it was observed that these lice were not resistant to other chlorinated hydrocarbon insecticides which had not been previously used in this area (Eddy, 1952). Similar observations were made on lice in Tokyo (Barnett and Knoblocks, 1952).

In Egypt, DDT powder was routinely applied for delousing as a public health measure at 3 to 5 month intervals between 1946 and 1951. DDT resistance was reported by Busvine in 1953 in a colony of body lice which had been established from the Cairo area in 1950 and maintained without contact with DDT for 2 years. Shawarby has reported on field tests conducted in Egypt in 1950. The data presented in the present paper were obtained between December 1951 and May 1953.

TESTS WITH DDT POWDER

Patch tests using adult lice from naturally infested persons living about 20 miles north of Cairo, are summarized in Table 1. Unfed lice were confined on stockinette dusted with about .003 gm. of powder per square centimeter for 24 hours at approximately 85°F. and 78 per cent relative humidity. The mortality averaged 31 per cent for 0.1 per cent DDT, 70 per cent for 1.0 per cent DDT and

* The study was conducted under the auspices of the Ministry of Public Health of the Egyptian Government and the U.S. Naval Medical Research Unit No. 3. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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TABLE 1

Patch tests with Egyptian body lice. Results in 24 hours with unfed lice on .003 gram powder per square centimeter, 85°F., 78% relative humidity. 4 tests, 40 lice per test

Concentration of DDT* Per Cent	Per Cent Mortality	Range Per Cent
0.1	31	20–58
1.0	70	50–83
10.0	89	83–100
Control	7	3–13

* LD_{50}: 0.33 per cent; slope: 13.6.

TABLE 2

Per cent mortality of unfed Egyptian body lice exposed to cloth dusted with 10 per cent DDT, and worn for various periods of time. 4 subjects, 40 lice each

Time Worn	Per Cent Mortality in 24 Hours	
	Average	Range
Fresh	89	83–100
1 day*	41	30–67
2 days	31	12–55
4 days	14	5–22
8 days	18	5–35

* Three 8-hour days.

89 per cent for 10 per cent DDT. Under similar conditions the mortality for Korean lice was 48 per cent with 10 per cent DDT powder (Hurlbut *et al*, 1952). According to published data 0.25 per cent DDT powder gives 100 per cent mortality in a standard laboratory strain (Bushland *et al*, 1945).

When stockinette sleeves dusted with 10 per cent DDT were worn on the upper arm by laboratory personnel for various lengths of time, toxicity was lost at the rate indicated in Table 2. The method of testing was the same as for the patch tests. After one day of wear the mortality was 41 per cent, after four days it was only 14 per cent. According to published data (Bushland *et al*, 1945), under similar conditions complete kills of a laboratory strain of lice were obtained for 30–40 days.

A field test[6] conducted on naturally infested subjects in the village of Tersa, Giza province, was performed to measure the effectiveness of DDT under practical conditions. This village of about 3,000 inhabitants was divided into three sections. One was treated with 10 per cent DDT powder, another with BHC containing 0.5 per cent of the gamma isomer and the third served as a control. Only people who were lousy were dusted. Hand operated dust guns were used to apply the powder without removing the clothing, in the manner described by Soper *et al*, 1947. The people were not confined or segregated so it was possible for reinfes-

[6] Technicians and nurses from the Giza Health Department gave valuable assistance through the courtesy of Dr. M. Anwar, Director of the Epidemic Control Section and Principal Medical Officer.

TABLE 3

Summary of louse counts in Tersa after dusting with 0.5 per cent BHC and 10 per cent DDT. A comparison is made with other villages in Giza in 1945

	Number of Individuals Examined	Per Cent Positive	Per Cent Heavily Infested (10 or More Lice)
Pretreatment			
BHC	80	100	36
DDT	80	100	56
Control	43	100	37
1945 results, Giza	76	100	21
Three days after dusting			
BHC	70	32	3
DDT	21	29	0
Control	30	80	40
One week after dusting			
BHC	76	57	11
DDT	77	39	3
Control	37	89	38
Two weeks after dusting			
BHC	75	59	12
DDT	76	37	7
Control	34	82	24
1945 results, Giza	76	13	0
Three weeks after dusting			
BHC	52	62	15
DDT	63	35	3
Control	39	69	33
Four weeks after dusting			
BHC	76	50	13
DDT	77	28	9
Control	43	77	37
1945 results, Giza	76	13	0

tation to occur. To provide a comparison of the work in 1945 with the present, groups of pretreatment positives from the same general locality were taken from 1945 records together with the associated post-treatment data. The results are presented in Table 3. It is evident that DDT is still effective in reducing lousiness. The percentage of heavily infested people (those harboring 10 lice or more) was reduced from 56 per cent before treatment to 9 per cent four weeks after treatment, while the control group remained approximately the same. The 1945 records indicate a distinctly greater reduction both in total numbers infested and in those heavily infested. BHC was less effective than DDT.

In another type of field test, 10 infested subjects were selected from the villages of Quaranfil and Aghur El Sughra, Qalyubia province, the total number of lice on each was counted and then the clothing of each individual was dusted with 10 per cent DDT. This was repeated on the same subjects once each month for five months. These people were not segregated in any way and so were exposed to reinfestation in a normal manner. However, the other inhabitants were dusted

TABLE 4

Results of monthly dusting of 10 heavily infested individuals with 10 per cent DDT

Subject Number	Per Cent Lice Killed by 1% DDT at Beginning	Total Monthly Body Louse Counts					
		(Pretreatment)	Jan.	Feb.	March	April	May
1	67	54 (48)*	1	20	0	12	69
2	78	122 (47)	50	57	—	†216 (30)	—
3	55	154 (66)	—	1	0	2	0
4	60	99 (60)	9	0	0	0	4
5	95	113 (61)	5	4	†76 (30)	88	—
6	90	165 (70)	69	37	0	—	20
7	60	67 (39)	0	2	3	12	0
8	61	139 (45)	14	0	2	0	6
9	50	159 (57)	7	47	†132 (30)	0	27
10	90	130 (60)	74	—	—	—	—
Total.....	135/190	1202	229	168	213	330	126
Av.....	71	120	25	19	27	41	18

* Figures in parentheses are number of adult lice collected for patch tests.

† Patch tests with 1 per cent DDT gave per cent mortalities as follows: No. 2—80; No. 5—50; No. 9—65.

at least once during the five months with BHC as a routine public health measure. During the first pretreatment count adult lice were removed and patch tests performed on them with 1 per cent and 10 per cent DDT according to the method already described. The data are presented in Table 4. The pretreatment patch test gave an average mortality of 71 per cent with 1 per cent DDT and 81 per cent with 10 per cent DDT. The mortality in the controls was 5 per cent. While a marked reduction occurred in the total number of lice, approximately one-half of the subjects remained heavily infested in spite of the treatment. No demonstrable increase in resistance to DDT was observed.

TESTS WITH CLOTH IMPREGNATED WITH DDT

Newly hatched nymphs from eggs deposited by females collected at Aghur El Sughra were placed on pieces of wool suiting which had been immersed for 10 seconds in acetone solutions of technical DDT of various concentrations. The lice were fed for about 30 minutes twice each day. At other times they were kept at about 85°F. and 78 per cent relative humidity. Two pieces of cloth 3 cm. square were used for each group of 100 lice. The lice were thus exposed to DDT continuously throughout their life cycle.

The data are summarized in Table 5. Seventy-six per cent of the lice on untreated cloth survived through the third and last nymphal stadium and molted to the adult stage. On the cloth which had been dipped in an acetone solution containing 1 gram of DDT per 10,000 ml., 56 per cent survived to the adult stage. The 1–5,000 concentration yielded 46 per cent, the 1–2,000, 2 per cent and the 1–1,000, no survivors. Under similar conditions Korean lice gave 35 per cent survival with the 1–1,000 concentration (Hurlbut *et al*, 1952).

TABLE 5

Survival of Egyptian body lice reared on cloth impregnated with DDT. 100 lice per group

Nymphal Stadium	No. Days Since Hatching	Per Cent Surviving				
		Control	*1–10,000	1–5,000	1–2,000	1–1,000
1	4–7	97	†83	69	22	9
2	8–12	86	71	59	7	1
3	13–17	76	56	46	2	0

LD_{50}, 1st instar: 1–3704; slope: 2.41.

* Cloth impregnated by dipping in acetone containing 1 gram of DDT to 10,000 ml. acetone.

† 83 per cent lived through the 1st nymphal stadium and molted.

TABLE 6

Per cent survival of body lice, from 5 localities in Egypt, reared on cloth impregnated with DDT from acetone solution, 1 part DDT to 5,000

Locality	Number Newly Hatched Nymphs	Per Cent Reaching Adult Stage
Aghur el Sughra	100	38
Aghur el Sughra	100	35
Helwan	66	11
Motia	59	7
Nagada	38	13
Aramant	100	13

The rearing technique on impregnated cloth was used to test lice from four other localities in Egypt, and from four localities outside of Egypt where previous contact with DDT was unlikely. Adult female lice were collected from naturally infested subjects. These lice were not fed but eggs deposited by them remained viable and hatched after 8–10 days. It was thus possible to send them to the laboratory in Cairo for testing. The results indicate that this method could be used for survey purposes. The testing could be done in a central laboratory on material sent a considerable distance by air. The mortality in lice of the first instar should furnish an adequate index of resistance. Data from the tests on Egyptian lice are presented in Table 6. Two additional samples from Aghur El Sughra are included. The apparent higher susceptibility of lice from the other localities more remote from Cairo may be due to less frequent contact with the insecticide. The two samples each from Cyrenaica and Sinai indicated a high degree of susceptibility to DDT, almost all being killed by contact with the 1–10,000 concentration. The data are presented in Table 7. It is probable that the Bedouins from whom these lice were collected had made little use of DDT.

The question of acquisition of resistance was investigated experimentally by rearing lice through six generations on cloth impregnated with DDT. Freshly impregnated cloth was used for each generation. The data are presented in Table 8. A self-propagating strain was maintained on a concentration of 1–5,000 for 2

TABLE 7

Survival of body lice from areas where previous contact with DDT is unlikely. Lice reared on cloth impregnated with DDT from acetone solution

Place Collected	Control	1-10,000	1-5,000
Cyrenaica			
Augdabia	34/45 = 76	6/7 = 0	0/40 = 0
Gardinia	11/12 = 92	1/33 = 3	0/30 = 0
Sinai			
St. Catherine's	—	0/29 = 0	—
Wadi Feiran	14/35 = 40	0/40 = 0	—

1st instar mortality on 1-10,000 conc.: 90-100 per cent.

TABLE 8

Lineal rearing of Egyptian body lice on cloth impregnated with DDT from acetone solution

Generation Number	Concentration of DDT in Acetone Solution					
	1-5,000		1-2,000		1-1,000	
	No. 1st instar	Per cent adults reared	No. 1st instar	Per cent adults reared	No. 1st instar	Per cent adults reared
1	100	*35	100	0		
2	100	*22	100	3		
3	100	69	100	*40		
4	100	42	200	*22	167	2.4
5			181	*29	200	3.5
6			200	22.5	310	0.6

* Parent group for all concentrations.

generations after which it was established on the 1-2,000 concentration and reared through 4 generations. Efforts to establish the strain on cloth impregnated with the 1-1,000 dilution failed although a few individuals survived to the adult stage. The authors served as blood donors during the six months period of this experiment. The strain was lost when an attempt was made to change to different donors who proved to be unsatisfactory hosts. The experiment has not been repeated.

DISCUSSION

The degree of susceptibility of Egyptian body lice to DDT appears to be intermediate between the extreme condition found in Korea and the state of high susceptibility which exists in lice from certain areas where previous contact with the insecticide is unlikely. While we lack definitive data on the primary susceptibility of body lice to DDT in areas where resistance now is evident, the available information seems to favor the view that resistance has been acquired through selective breeding brought about by killing off the more susceptible lice.

It is of interest to consider the environmental conditions which appear to operate in the acquisition of resistance. One of the most important effects of the high susceptibility which was early observed (Bushland *et al*, 1945) was the pro-

longed residual action of the traces of DDT which remained for weeks in clothing treated with DDT powder. The observations on Egyptian lice suggest that this high susceptibility has been lost so that newly hatched nymphs, always the most potent source of reinfestation, are largely unaffected when they hatch 3–4 days after treatment. It is in this post-treatment environment that conditions favorable for selective breeding exist. Over a period of 8–10 days after the DDT is applied, hatching nymphs emerge into an environment which presents a gradient of decreasing concentration of the insecticide. An indication that selective breeding does result in increased resistance is found in the experiments which have been described.

In view of these findings the advantages and probable disadvantages of routine use of DDT for delousing should be carefully weighed. It may be advisable to reserve the use of DDT and presumably other related compounds for emergencies when there is imminent danger of typhus and to avoid large scale routine use over a long period of time.

SUMMARY

1. The average mortality for adult Egyptian body lice from the vicinity of Cairo when exposed to DDT powder for 24 hours was 31 per cent for one tenth per cent DDT, 70 per cent for one per cent DDT, and 89 per cent for ten per cent DDT, giving an LD_{50} of 0.33 per cent. After four days of wear, sleeves which had been treated with 10 per cent DDT killed only 14 per cent of the lice exposed to them.

2. The results of a field test showed that 10 per cent DDT powder is still effective in reducing lousiness. The percentage of heavily infested people (harboring 10 lice or more) was reduced from 56 per cent before treatment to 9 per cent four weeks after treatment while the control group remained approximately the same. When 10 very heavily infested people were treated once each month for five months the number of lice was markedly reduced but about half remained relatively heavily infested.

3. Tests on cloth treated by dipping in acetone solutions of DDT showed that Egyptian body lice could be propagated on cloth which had been treated with a 1–5,000 dilution of DDT. After two generations these lice were established on cloth treated with a 1–2,000 dilution and maintained on it for four generations. The 1–10,000 dilution proved to be lethal to lice from four localities where it is unlikely that there had been previous contact with DDT.

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PRIVIES AS A SOURCE OF FLY PRODUCTION IN AN URBAN AREA

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It has long been recognized that exposed human excrement may serve as a source of disease pathogens which can be carried by insect vectors to the residents of a community. It is likewise a matter of common observation that muscoid flies are attracted by and can usually gain access to human excrement in privies despite man's efforts to build these structures so as to prevent fly entry. That flies do breed in human excrement was reported by early workers (Howard, 1900; Hewitt, 1914) during the period when the privy was a standard structure in most communities. Since that period, remarkable progress has occurred in the field of disposal of human wastes but even today there are localities in which privies still are utilized to a considerable extent. In such communities, privies can represent a significant source of fly production for house-frequenting species.

To evaluate the importance of the problem in the Phoenix, Arizona, area, privy surveys were conducted in the fringe metropolitan areas outside the city during 1951–52. This paper describes the findings of that investigation.

METHODS

The majority of the privy samples were obtained from a sub-standard area which was composed of 273 residential blocks located adjacent to the southern city limits. During the course of surveillance, a total of 270 privies was checked (approximately a 12 per cent sample) on a schedule of 10 samples every two weeks.

To obtain the samples, an ordinary enamel water dipper (225 ml. capacity) bolted to a five-foot length of wooden handle was used. A sample of the excrement was withdrawn from the privy pit and carried to the exterior. Larvae present in the sample were then removed with forceps and placed in plastic vials containing preservative fluid. As a rule, 3 to 10 samples were made at each privy, the greater number being required to establish the negative state of the vault contents. A sufficient number of larvae was taken from each infested substrate to yield a representative sample of the different larvae present. Field records were maintained to show the location of the sampling site, an approximation of the cubic feet of infested medium, and the degree of infestation.

Field collections were brought to the laboratory for determination. The larvae were identified as to genera by the use of taxonomic keys (James, 1947; Peterson, 1951). To check the accuracy of difficult determinations, specimens occasionally were reared to the adult stage.

The privies examined were of the pit type with depths in the range of two to six feet. Construction of the privies varied from well-enclosed structures to those of loose framing supplemented by sacks or roofing paper. In no sense could any of the privies be classed as fly-tight or flyproof. Vault contents ranged from

solid to liquid in consistency. In some instances, the householder also utilized the privy as an auxiliary disposal site for garbage.

As an adjunct to the urban survey, a number of privy samples were also obtained by the same technique from a nearby village. The latter was located approximately three miles from the urban area, part of the intervening distance being a mile expanse of dry river bed.

RESULTS

Data obtained from the urban study are summarized in Table 1. With reference to this table, it may be noted that there is no marked difference in the percentage of privies infested by fly larvae during spring and summer. The inincreased percentage of privies infested in the fall is offset by a comparable reduction in the winter, a period when a decrease in fly production would be expected because of lower temperatures.

The greatest seasonal fluctuations are evidenced in the generic composition of positive samples. On a yearly basis, more samples were positive for *Musca domestica* than for any other type of fly. However, the incidence of *Sarcophaga* and *Muscina* exceeded *Musca* in the spring and winter. During the fall months, *Sarcophaga* infestations were slightly in excess of those of *Musca*.

Phaenicia spp. are not regarded generally as excrement breeders. However, Thomsen and Hammer (1936) report eggs and larvae of *P. sericata* in pig excrement. Both *Phaenicia* and *Calliphora* larvae are more commonly found

TABLE 1

Fly larvae identified from privy samples obtained in an urban area. Phoenix, Arizona, 1951–52

Period	Number of Privies		Percentage of Positive Samples Yielding:							
	Checked	Positive	Musca domestica	Phaenicia*	Sarcophaga	Muscina†	Calliphora	Ophyra‡	Hermetia§	Others
Spring (1952) (Mar.-Apr.-May)	66	50 (75%)	12.0	6.0	16.0	34.0	0	4.0	2.0	0
Summer (June-July-Aug.)	75	58 (77%)	74.1	0	20.7	5.2	0	8.6	12.1	5.2
Fall (Sept. - Oct. - Nov.)	60	53 (88%)	56.6	0	58.5	3.8	0	9.4	11.3	7.5
Winter (Dec. - Jan. - Feb.)	69	32 (46%)	21.9	0	28.1	28.1	3.1	9.4	21.9	3.1
Total.....	270	193 (71.5%)	44.6	1.6	31.1	16.1	0.5	7.8	10.9	4.1

* Two samples presumably *P. sericata*; one, *P. pallescens*.

† Presumably *M. stabulans*.

‡ Presumably *O. aenescens*.

§ *Hermetia illucens* is the predominant Stratiomyid in this area.

breeding in animal matter than in excrement. Species of the latter genus are present in Phoenix from November through April, but are notably absent during the warm months of the year.

A few of the positive samples contained representative species of Anthomyiidae, Drosophilidae and Syrphidae, but their incidence was too infrequent to warrant their specific inclusion in the table. They are listed as "other."

Twelve or more species of *Sarcophaga* are known to be indigenous to the study area. This group commonly utilizes excrement as breeding media, hence it is not surprising that they appeared second only to *Musca* in the utilization of human excrement as a production source.

Ophyra larvae were observed frequently in various types of animal excrement in the Phoenix area. Larvae of this genus appear to be particularly well adapted for development in rabbit excrement.

Table 2 presents a comparison between the data obtained from privy samples in the two study areas, urban and village. Unfortunately, time did not permit the collection of privy samples from the village area during the summer period. However, comparable data are available for the remaining seasons of the year.

It will be noted from Table 2 that species composition of privy samples was quite different in the two areas. The village environment reflected a dominance of two types, *Muscina* and *Hermetia*, whereas the city environment showed a more even distribution of the different species of flies involved. The fact that *Muscina* predominated over *Hermetia* in spring and winter whereas the reverse was true in the summer and fall (Table 1) exaggerates the dominance of *Muscina* in the village area during the study period. From infestation rates in June, July and August, *Hermetia* larvae appear to be more tolerant of extreme conditions and better adapted for summer survival than *Muscina*.

The condition of the vault contents influences the types of flies produced therein. In this study, few flies other than Syrphidae were recovered when the pit contents approached a watery consistency. In contrast, soldier flies (*Hermetia*)

TABLE 2

Comparison of privy survey data obtained from two different type study areas; fall, winter, and spring, 1951–52

	Urban	Village
Number samples obtained	195	102
Number samples positive	135	83
Percentage samples positive	69.2	81.4
Percentage of positive samples, yielding:		
Musca domestica	31.9	3.6
Phaenicia	2.2	0
Sarcophaga	35.6	10.8
Muscina	20.7	59.0
Calliphora	0.7	1.2
Ophyra	7.4	4.8
Hermetia	10.4	44.6
Others	3.7	6.0

were more abundant in semidry media. House fly larvae were recovered frequently from moist feces protected by an outer insulating crust of excrement. In many instances, the maggots were found between layers of newspaper or tissue.

Despite the varied depths of the privy pits surveyed, the present work gave no definite assay of the relative infestation rates in "dark" versus "light" privy vaults. However, it was evident that *M. domestica* did infest human feces in vaults under conditions which were considered of low light intensity.

DISCUSSION

In attempting to associate flies with the spread of poliomyelitis, certain workers have indicated that blow flies are more readily attracted to human stools than is *M. domestica*. Data from trapping studies in Tennessee and Michigan (Francis *et al.*, 1948) favor this viewpoint. However, the present findings reveal the house fly to be a significant element of the fly population produced from privies, a situation which provides ample opportunity for fly contamination with pathogens present in the feces. Obviously, house flies must be attracted to feces, otherwise they would not infest such a medium so readily. At least in the Phoenix, Arizona, area, *Phaenicia* spp. apparently contact human stools primarily for feeding purposes since they do not appear to utilize this medium as a frequent breeding source.

The high infestation rates reported for *M. domestica* must be viewed with reference to the locale involved. In the hot, dry climate of the southwestern United States this species is predominant and infests almost every type of available substrate. Because many substrates become desiccated in the summer, human excrement in privies represents one of the few media that offers a moist environment throughout the year.

In the few well-constructed privies encountered on the surveys, ample opportunity still existed for fly entrance through cracks, unlatched doors, or ventilating louvers. After having examined numerous privies in Phoenix and in other areas, the writers feel that few, if any, such structures ever remain flyproof even though the original construction may have been reasonably fly-tight. From the fly control viewpoint, the only positive answer to eliminating fly production in human excrement apparently is to prevent the flies from gaining access to the fecal material by replacement of the privies with sewerage systems, septic tanks, or other flyproof means.

As shown (Table 2), distinct differences existed in the infestation rates of the various species produced from privies in the two study zones. *Musca domestica* infested over 30 per cent of the privies in the city area as compared to only 3.6 per cent in the village. The latter percentage conforms to the opinion current in the period 1945–50 that few house flies breed in privies (Lindsay[1]; Quarterman *et al.*, 1949). However, in 1951, copious house fly breeding was detected in chemically treated privies at Yuma and Phoenix, Arizona, and was also reported from

[1] Personal communication from Dr. D. R. Lindsay regarding unreported studies in Rio Grande Valley, Texas, 1946–48.

TABLE 3

Comparison of urban and village study areas

	Urban	Village
Population (1950 census)	75–150 persons per block	25–50 per block
Racial composition	Latin, negro, white	Negro
Housing	Mixed business, a few stores, shops and industries	Strictly residential
Animal pens	Numerous	Few or lacking
Garbage disposal practices	Inadequate, open dumps, burning, privy pits sometimes used	Inadequate, open dumps, burning
Fly densities	High	Moderate to low

the Rio Grande Valley, Texas[2]. The Texas workers suggested that the contrast between these data and those of 1946–1948 might be an effect of chemical control measures. As a result, the Phoenix studies were undertaken to compare the village area which represented an untreated zone with the urban section which had been subject to insecticidal treatment.

Comparison of the data from the two zones (Table 2) would appear to justify the assumption that the application of insecticides (dieldrin) had modified the species composition of the privy fly populations in the urban area, particularly as it related to *Musca domestica* and *Muscina*. However, examination of the other factors tend to lessen the strength of such an assumption. First is the fact that the last chemical treatment of the urban privies occurred in early 1950 so that the term "treated privies" must be received with reservation. A second factor concerns differences in the environmental make-up of the two study areas (Table 3). It is obvious that the urban area is more representative of copious fly attractants and production media, and greater fly densities that is the village area. Because of the unknown effects of these factors, it is not possible with the present data to ascribe the increased rate of infestation of *Musca domestica* in the "treated" privies to be solely the result of chemical treatment.

The evidences which support the "chemical" treatment hypothesis are the known occurrence of dieldrin-resistant house flies in the urban area and the relatively low frequency of *Muscina* infestations in the treated urban privies as compared to those in the village privies. As adult *Muscina* spp. have not been shown to develop resistance to chlorinated hydrocarbons, their ability to invade and establish infestations in treated structures would be reduced materially. In turn, since *Muscina* larvae are predacious upon *Musca domestica* larvae (Portchinsky, 1913)[3] the resulting decrease in predation would be conducive to an increase in the extent of house fly breeding in the treated privies.

[2] Kilpatrick, J. W., and M. D. Bogue. (Unpublished manuscript.) Adult fly production from privy pits in the lower Rio Grande Valley of Texas. Communicable Disease Center, Savannah, Georgia.

[3] Laboratory tests by writers with artificial media indicated that cultures seeded with known numbers of eggs of *Musca* and *Muscina* showed adult emergence rates of 2.2 and 44.9 per cent respectively as compared to 68 per cent for each genus when the larvae were

Further circumstantial evidence supporting the insecticide viewpoint is found in data from Charleston, West Virginia, where limited surveys showed heavier *M. domestica* breeding in the treated privies, and from Texas[4].

In summation, the predation of *Muscina* larvae upon the larvae of *Musca domestica*, the reversal of the infestation rates of *Muscina* and *Musca domestica* in treated and untreated privies, and the similar occurrence of heavy house fly breeding in treated privies in several widely scattered localities represent data which favor the premise that chemical treatment of privies may alter the species composition of fly populations produced in the vaults. Nonetheless, in the face of differences present in the environmental characteristics of the two study zones, the findings for the Phoenix area cannot be considered conclusive substantiation of this premise. Such proof must await studies wherein the relative influence of the various environmental factors can be positively assessed.

SUMMARY

1. Surveys of human excrement in privies at Phoenix, Arizona, during 1951–52 demonstrated that *Musca domestica* breeds readily in this medium. Of 193 privies positive for fly breeding, 44.6 per cent were infested with house fly larvae. Larvae of *Sarcophaga*, *Muscina*, and *Hermetia* occurred in 31.1, 16.1 and 10.9 per cent of the positive samples, respectively.

2. Samples of human excrement from chemically treated privies in an urban area showed infestation rates of 31.9 per cent for *M. domestica* and 20.7 per cent for *Muscina* spp. whereas those from privies in an untreated zone displayed rates of 3.6 per cent and 59.0 per cent for *M. domestica* and *Muscina* respectively. No conclusive data explain this reversal in species predominance but several possible causes are discussed.

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maintained in separate cultures. *Muscina* also was shown to prey on larvae of *Sarcophaga* and *Phaenicia pallescens*.

[4] In their Texas studies, Kilpatrick and Bogue also stated that *M. domestica* in the treated town was highly resistant to dieldrin; in the untreated areas, the early tests revealed minimum resistance to dieldrin but the resistance level gradually increased concomitant with an augmentation of house fly production from the privies.

A SURVEY OF 200 CASES OF SNAKE-BITE IN VIRGINIA

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Snake venom poisoning has long interested physicians. Case studies were so frequent in the medical journals of the nineteenth century that Willson (1908) was able to compile 440 published reports of snake-bite accident in the United States. The educational and preventive aspects of the problem of ophidian venenation were not neglected in this period, and the outstanding contributions of Ellzey (1885) and Barringer (1892) focused attention on the circumstances occasioning bites. The magnitude of the snake-bite problem in the United States was first quantitatively investigated by Hutchinson (1929, 1930). He reported that in 1928 four per cent of the 607 cases that occurred in that year in the United States had taken place in Virginia. Githens (1935) extended this work on the national incidence of snake-bite, and on the basis of reports returned by physicians to the Antivenin Institute of America, plus newspaper records, he estimated an annual morbidity rate of from one to two thousand cases. To date no investigation has provided adequate data on either the incidence in the nation, or in any region therein.

Reported here are compiled data from 200 cases of snake-bite that occurred in Virginia in recent years (98.5 per cent are from the 13 year period, 1941 through 1953). No critical estimate of the annual incidence of snake-bite in this region is possible from these data because they are based primarily on hospitalized cases. It is appreciated that in this period many cases were neglected, received self-treatment, first aid, or ambulatory treatment by a physician in the victim's home, the doctor's office, or a hospital emergency room, and no records of these cases are available. Furthermore, the hospitalized cases were obtained from 19 general hospitals comprising only one-third of the general hospital beds in the state, and even in these institutions the systems used in the record departments have changed during the period of this study, with the result that records are fairly complete in only the three most recent years (1951–53) when most institutions were using the standardized nomenclature of disease.

METHOD OF SURVEY

This survey is based upon a study of the medical records of 196 persons hospitalized following injuries by venomous snakes, and four other reports including two mortalities due to snake venenation provided by the Department of Vital Statistics, Commonwealth of Virginia (A. R. Thomas; personal communication, Sept. 3, 1953), an additional and earlier mortality record (O. K. Goodwin, personal communication, Sept. 8, 1953), and a detailed report of a copperhead bite that occurred in the Norfolk area, and was treated in a New Jersey hospital (E. E. Buckley, personal communication, July 14, 1953). The

hospitalized cases were located by writing to 95 general hospitals in Virginia, requesting data on the incidence of morbidity and mortality attributed to snake-bite accidents, as shown by their records from 1943 through 1952. Data were obtained from 40 hospitals, but records were available for study in only 19. Medical records were studied in the following Virginia hospitals: Bell Hospital, Chesapeake and Ohio Hospital, Clinch Valley Clinic Hospital, DePaul Hospital, Gordonsville Community Hospital, Johnston Memorial Hospital, Lee General Hospital, Lynchburg General Hospital, Medical College of Virginia Hospitals, Martha Jefferson Hospital, Mary Washington Hospital, Norfolk General Hospital, Rockingham Memorial Hospital, St. Mary's Hospital, United States Naval Hospital (Quantico), University of Virginia Hospital, and Warren Memorial Hospital. Additional information was obtained by discussions with physicians in these hospitals who had provided the data for the medical records, and interviewing patients. Medical records were also obtained from Northampton-Accomack Memorial Hospital (E. S. White, personal communication, June 17, 1953) and Cora B. Miller Hospital (Dr. H. W. Miller, personal communication, Aug. 11, 1953).

As many as possible of the following points were obtained from the medical records: (1) age, sex, and race of the patient; (2) date of injury, and the conditions under which it occurred; (3) species of venomous snake involved; (4) anatomical site of the injury; (5) extent and type of pre-hospitalization therapy; (6) signs and symptoms on admission to the hospital; (7) emergency room definitive and supportive therapy; and (8) hospital course including complications and sequelae when present.

EPIDEMIOLOGY OF SNAKE-BITE

Distribution of snake species. The copperhead is ubiquitous in its distribution in Virginia, being widely distributed in the three chief physiographic regions of the state (Coastal Plain, Piedmont, and Valley and Ridge Province). The distributions of the larger and more dangerous venomous species are confined to more restricted geographic ranges. The timber rattlesnake inhabits the hills and mountains comprising the western half of the state, being more abundant on the higher slopes. The cane brake rattlesnake and cottonmouth moccasin are found only in the southeastern counties of the Coastal Plain. The cane brake rattlesnake has the more extensive range of these two species, being present on the lower York-James peninsula in the cities of Hampton and Warwick, and possibly in Newport News, as well as in most counties south of the James River and east of Petersburg. The cottonmouth is found only south of the James River and east of Petersburg, and is common in only a few habitats in the three easternmost counties, in the Dismal Swamp and its periphery.

Distribution of snake-bite accidents. Medical records mention the species of snake responsible in 134 cases of venenation, as follows: copperheads (*Ancistrodon contortrix mokeson*), 119 cases; cottonmouths (*Ancistrodon p. piscivorus*), 1 case; timber rattlesnakes (*Crotalus h. horridus*), 12 cases; and cane-brake rattlesnakes (*Crotalus horridus atricaudatus*), 2 cases.

TABLE 1

Seasonal distribution of 190 snake-bite cases in Virginia, (1943–1953)

Month	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Number of cases	0	0	0	3	18	31	60	36	37	5	0	0
Per cent of cases	0	0	0	1.6	9.5	16.3	31.6	18.9	19.5	2.6	0	0

Although venomous snakes are found in all parts of Virginia, a higher incidence of accidents occurs in the mountain and valley area than in the Piedmont and northern Coastal Plain, with the lowest incidence on the southern Coastal Plain; snake-bite accidents are in the proportion 3:2:1 in these areas respectively.

Analysis by sex and race. The ratio between white and colored hospitalized patients in this series is 9:1 (172 white; 19 colored); in Louisiana the ratio is approximately 2:1 (Swartzwelder, 1950). The ratio of male to female victims is 7:3 in the United States (Hutchinson, 1929), 5:1 in Louisiana, and 2:1 in this Virginia series.

Influence of season and time of day. The seasonal distribution of snake-bite accidents in the United States (Hutchinson, 1929; 1930) has little in common with that in Virginia (Table 1) for the reason that broad geographic studies include regions having very different climates. The snake poisoning danger is related to the season of activity of the venomous snakes in a specific area, which depends on the extent of the hibernating and estivating intervals. In Virginia 96 per cent of the cases of snake-bite fall in a five month period from May through September, with a peak incidence of 32 per cent in July. Only four per cent of the Virginia bites are reported in the seven-month period from October through April, an interval in which 38 per cent of the Louisiana bites are recorded. Only 10 per cent of the Virginia venenation cases occur in May when the snakes are emerging from their hibernating and courting sites, and are relatively remote from rural dwellings, while 20 per cent of the bites occur

TABLE 2

Snake-bite accidents in Virginia as related to time of occurrence, (1943–1953)

	Number of Cases	Per Cent	Total per Cent
Diurnal hours:			67.3
6 AM– 9 AM	6	5.6	
9 AM–12 AM	30	28.1	
12 AM– 3 PM	16	14.9	
3 PM– 6 PM	20	18.7	
Nocturnal hours:			32.7
6 PM– 9 PM	21	19.6	
9 PM–12 PM	11	10.3	
12 PM– 3 AM	2	1.9	
3 AM– 6 AM	1	0.9	

during September when the snakes are common in the vicinity of farms where the foraging for small rodents is rewarding.

In Virginia most bites occur during the months when the snakes are active at night and in concealment by day. Nevertheless two bites occur during daylight hours for every one at night (Table 2). Most snake bites received at night are on the lower extremities while diurnal bites are more frequent on the upper extremities. Nocturnal bites are received from snakes encountered out of concealment, frequently from snakes that have been stepped on. The copperhead is chiefly active at night (Swartzwelder, 1950) and in Indiana the majority of bites seem to occur between the hours of six and nine in the evening (Minton, 1950). Diurnal bites occur when snakes are accidentally exposed in their places of concealment under logs, stones, boards, thick bushes, piles of hay and debris heaps. Diurnal bites by snakes out of concealment occur in places that simulate a nocturnal environment such as dark cellars, sheds, caves, mines, tunnels, and under porches and houses.

Relation of age to incidence and location of bite. The relationship between accident incidence and the age of the victim is provided in Table 3. Approximately half of the bites are received by persons under 16 years of age: in Florida, 46 per cent (Allen, 1953); Virginia, 52 per cent; and the United States, 45.6 per cent (Hutchinson, 1929). In Louisiana the number of cases involving victims under 16 approximated that of the young adults (16 to 30 years). This was not true in Virginia where a sharp reduction in snake-bite accidents was found in the persons over 15. In Virginia the youngest children are in the greatest danger of receiving snake-bites, and it is reported that this group shows the greatest morbidity (Willson, 1908), which emphasizes the necessity for better information on the circumstances under which bites occur and for preventive measures.

The body area sustaining the bite is related to the severity of poisoning and the mortality rate (Willson, 1908). Fatalities from upper extremity bites are twice as numerous as those from the lower extremities. Head and trunk bites are from two to three times as dangerous as those on the hands and arms. In four studies in the United States the precentage of bites on the upper limb are reported as 41.0, 41.2, 42.2, and 43.6; those on the lower limb comprise 54.1, 55.2, 58.0, and 58.5 per cent. Head and trunk bites range from 0.5 to 3.7 per cent in these series (Willson, 1908; Hutchinson, 1929; 1930; Swartzwelder, 1950). In Virginia there are marked differences in the distribution of bites. The total upper extremity bites comprised only 33.5 per cent of the wounds, and the

TABLE 3

The relationship between the age distribution and the frequency of hospitalized cases

	Number of Cases	Per Cent of Cases		
		Under 16 years	16 to 30 years	Over 30 years
United States (Hutchinson, 1929)	535	45.6	26.0	28.4
Louisiana (Swartzwelder, 1950)	301	36.2	34.0	16.0
Virginia	190	52.0	18.5	29.5

TABLE 4

The relationship between the location of the snake-bite and the age of the patient

	2 to 8 Years		9 to 15 Years		16 to 84 Years	
	No. of cases	Per cent	No. of cases	Per cent	No. of cases	Per cent
Upper extremity:		19.2		19.2		48.8
Fingers	3		4		22	
Hand	6		5		20	
Wrist	0		0		1	
Forearm	1		0		1	
Upper arm	0		1		0	
Lower extremity:		80.8		78.9		48.8
Toes	5		2		4	
Foot	20		19		15	
Ankle	10		16		13	
Shin	5		2		10	
Thigh	1		1		1	
Head and trunk:		0.0		1.9		2.4
Head	0		0		1	
Trunk	0		1		1	

lower limb received 65.4 per cent of the 192 cases in which the involved body area was cited. The plotting of the body area data against the age of the victim revealed for the first time a marked difference by age groups. In Table 4 a close similarity in the locations of bites exists among patients from two to 15 years old, with approximately 20 per cent of the bites on the upper extremities, and 80 per cent on the lower. In the cases involving persons over 15 years, however, the number of bites received on the upper and lower extremities are equal. Most of the bites received by young persons are not the result of reaching into places where snakes are in concealment; they are received while stepping on or near snakes in deep grass and weeds, or in playing around the home. The hazards of playing in deep grass around the rural home are increased during evening and night hours.

TREATMENT

In 168 cases of ophidian venenation on which therapy data are available it was noted that 90 per cent of the patients received antivenin. Those patients who were treated without the use of the specific antiserum were either strongly sensitized to horse serum as evidenced by positive skin tests, or were treated in areas where the antivenin was unavailable. One ampoule of 15 cc. of antivenin was administered to 88 per cent of the patients; the others received from 10 to 75 cc. No fatalities were recorded in the hospitalized series of patients, but there was a distinct clinical impression that the incidence of urticaria, pruritus, and angioneurotic edema was higher in those patients receiving the horse serum preparation.

PREVENTION

A survey of the circumstances under which snake-bite accidents occur is essential in the planning of appropriate measures to reduce the incidence of this type of animal poisoning. Hutchinson (1930) has summarized the circumstances in five groups as follows: picking up objects; reaching into or under objects; stepping on, near, or falling on snake; catching and handling snakes; and sleeping outdoors. His classification is of some merit in this Virginia series only in regard to cases involving victims over the age of 16. A survey of the environment in which the accidents occur (Table 5) shows that most small children (2 to 8 years old) are bitten near their homes or on farms near their homes. The activities of the children immediately prior to the accidents include playing on woodpiles, in deep weeds, under the porch, and near thickets. Children picking berries comprised the source of most of the venenations in this age group that took place on farms. One child was bitten while playing in the basement of an abandoned school house, and another was bitten while visiting a public recreation site.

More than one-fourth of all snake bites in this series involve children of eight or less years; most of these cases involve bites on the lower extremity. These facts, plus the knowledge that the accidents occur near the children's homes, indicate that control of venomous snakes near rural homes and on farms is required to reduce the incidence of these injuries. In the majority of cases copperheads are the responsible species, and steps can be taken to eradicate them from the vicinities of homes (Gloyd, 1944; Minton, 1950). Snakes become abundant where they find adequate shelter and an abundant food supply. They eat mice, shrews, and caterpillars (Uhler, Cottam, and Clarke, 1939), and on farms mice and other small rodents comprise the bulk of their food. Control of the small rodent population about dwellings is facilitated by removal of litter and suitable garbage disposal. Destruction of the rodents with poisoned grain is a useful technique in the control of the food supply of local venomous snakes. It is important to keep grass and weeds cut short in areas where infants and small children play, and in addition the shelter of the snakes should be reduced to a minimum near houses. "Snakes commonly take shelter beneath loose slabs of rock, boards, piles of bark, brush, logs, fence posts, straw, leaves, stones, old roofing paper, corrugated iron, burlap, paper cartons, and miscellaneous debris of all kinds," (Gloyd, 1944). "The cleaning up of such habitats

TABLE 5

The relationship between the age of the patient and the circumstances attending snake-bite

Age of Patient	Number of Cases	Activity or Environment (Per Cent of Cases)			
		Near home	On farm	Camping and hiking	Handling snakes
2 to 8	16	62.5	31.2	6.3	0.0
9 to 15	17	17.6	41.2	41.2	0.0
16 to 84	44	22.8	45.6	9.0	22.8

will greatly reduce the incidence of snakes about dwellings. It is safer to remove boards and other litter during the winter or early spring when snakes are inactive; however, a campaign during midsummer may dispose of gravid females before the young are born," (Minton, 1950). Several serious cases of venenation have resulted of bites from recently born juvenile copperheads described by their victims as "about six inches long", indicating that the proximity of a brood of young poisonous snakes to a children's play area is a real safety hazard.

Among children from 9 through 15 the majority of snake-bite accidents occur in the woods and fields, in areas remote from their homes. Approximately 40 per cent of the bites occur in the field, and another 40 per cent on hiking and camping trips. Activities associated with venenation accidents include berry-picking, loading hay, packing peaches, and pitching straw. Since the majority of the bites were located on the lower extremities, it is probable that stepping on or near the snakes preceded many of the accidents. In view of the impracticability of elimination of the food for snakes or their shelter in the field, perhaps the most useful step in reducing the incidence of snake-bite accidents in this age-group is through safety education programs in the public schools, with particular advice in regard to wearing protective clothing, and not putting one's hands and feet in places where one cannot see.

Two of the cases involving juveniles are of particular interest. A camping party near Luray decided to sleep in a barn, and shortly after retiring one of the campers complained of a wasp sting on her right arm. About two hours later another member of the party was awakened by a stinging sensation on her thigh. A flashlight revealed the presence of a copperhead nearby, and examination of both wounds showed the deep twin punctures made by fangs of such a snake. Both victims were hospitalized, and showed minimal evidences of venenation. It is probable that the copperhead was foraging, detected the presence of a warm object with its "heat-receptor" pits, and struck, injecting sufficient venom to immobilize its usual prey. Many of the nocturnal bites may occur in such a manner during the hunting activities of the snakes.

Cases of snake-bite among older persons (16 to 84 years) are often associated with occupation. Accidents while working on the farm, logging, trapping, cleaning ditches and sewers, picking apples, and cutting and mowing are common. The proportion of bites attending camping and hiking is diminished in relation to the proportion of the adult's time they occupy. Accidents around the home are again important since much adult activity is centered there. One new category appears in this age group, the persons intentionally handling dangerous snakes, including scientists, showmen, collectors, religious fanatics, and overconfident amateurs.

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Entomology (Medical & Veterinary) Including Insecticides & Insect & Rat Control, by D. N. Roy, M.D., D.T.M., D.Sc., Director and Professor of Medical Entomology, School of Tropical Medicine, Calcutta (Retd.), In-Charge of the Department of Medical Entomology & Biology, Indian Institute for Medical Research, Calcutta; and A. W. A. Brown, M.B.E., Ph.D., Professor and Head of the Department of Zoology, The University of Western Ontario, London, Canada, author of "Insect Control by Chemicals." Second Edition: 413 pp., illustrated. Calcutta: Excelsior Press. 1954. Price Rs.30, 45s., $8.

Laboratory Techniques in Rabies. WHO Monograph Series, No. 23, by various authors. 150 pp., 51 illustrations, with 1 in color. Geneva, Palais des Nations: World Health Organization. 1954. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Price £1, $3, Sw. fr. 12 (paperbound), or £1.5s., $4, Sw.fr. 16 (clothbound). French edition in preparation.

Protozoology, by Richard R. Kudo. Fourth Edition: 966 pp., 376 illustrations. Springfield, Ill.: Charles C Thomas. 1954.

BOOK REVIEWS

Amebiasis, American Lecture Series No. 191. A monograph in *American Lectures in Internal Medicine* by ERNEST CARROLL FAUST, Head of the Division of Parasitology, Department of Tropical Medicine and Public Health, Tulane University, New Orleans, Louisiana. 154 pp. illustrated, with one plate in color. Springfield, Illinois: Charles C Thomas, and Blackwell, Ltd., Oxford, England and Ryerson Press, Toronto Canada. 1954. Price $4.75.

This concise monograph on infection with *Endamoeba histolytica* presents lucidly the knowledge and viewpoint which Dr. Faust has acquired in his long study of the subject. The six chapters deal with the etiology, natural history, pathology, clinical manifestations, diagnosis, treatment and control of the infection. Each phase of the subject is dealt with in accurate detail, based upon the author's own observations or those of other workers, and supported by a bibliography of 219 references.

A few details are worthy of special note. In the section on modes of transmission emphasis is given to fomites, especially soiled clothing, in ignorant rural families and in children's asylums and mental hospitals. Water, food and flies are also duly emphasized. The development of the tissue lesions in the colon and elsewhere is accurately described and well illustrated. The clinical manifestations ascribed to amebiasis include an "asyndromic" group, and hepatomegaly in children, which this reviewer believes may be erroneously diagnosed as amebic because of inaccurate examination of intestinal contents. The most approved methods of demonstrating *E. histolytica* are given, except that no mention is made of Ritchie's formol-ether concentration technique, which many workers now prefer to zinc sulfate flotation. The discussion of chemotherapy is well balanced between the older amebicides and the antibiotics. Fumagillin, the latest addition to chemotherapy, is mentioned without detailed dosage. The chapter on "Control" vigorously urges application of measures which would lower incidence of infection from all sources. These measures include periodic examination and possible periodic mass treatment of public food handlers. Effective chemotherapeutic control in mental institutions is described. The need for prevention of infection in military personnel in tropical warfare is emphasized by a criticism of the Preventive Medicine Service of the U. S. Army for apathy to the importance of amebiasis at the beginning of World War II.

Dr. Faust's object was to provide a comprehensive picture of amebiasis as a clinical and public health problem, and he has achieved this admirably. The book is well printed and contains very few editorial or typographical errors. It is highly recommended to every student of the subject.

HENRY E. MELENEY

An Introduction to Pathology and Bacteriology for Medical Students in the Tropics by E. C. SMITH, B.A., M.D., D.P.H., Sc.D. Dubl., M.C.R.C.P. Lond., sometime Senior Pathologist, Medical Laboratory Service, Nigeria.; AND R. KIRK, O.B.E., B.Sc., M.D., D.P.H., F.R.S. Edin., Professor of Pathology in the Kitchener School of Medicine, University College of Khartoum. Second Edition. 390 pp. with 78 figs. and 18 colored plates, London, Staples Press 1953.

Although the title indicates that this book is written as an introductory text in pathology and bacteriology, the subject matter in "bacteriology" actually covers the whole field of infectious agents that cause diseases of man. The authors state that it is intended for medical students in the tropics but it includes little information that is not usually taught in courses in general pathology and infectious diseases in the best medical schools in temperate regions. Basic facts in pathology, bacteriology, virology, mycology and animal parasitology are presented in a remarkably clear and concise manner. Practically every sentence is a precise summary of accurate and important information. For so small a book covering such an extensive field, the illustrative material is also well chosen. Appropriate appendices cover

Types of Lymphadenopathy, Postmortem Technique, Stains and Culture Media, and a brief but representative list of references book.

Among omissions that might profitably have been included are (a) a comparison of the severity and variations in expressions of clinical disease caused by organisms that occur both in the tropics and in temperate zones, e.g., *Corynebacterium diphtheriae* and Beta streptococci; (b) a discussion of the importance of the thick blood film in diagnosis of such diseases as malaria and filariasis, and (c) mention of the differentiation of the Paracolon bacteria from the Coliform and Salmonella groups.

This book should serve advantageously as an outline for review or as a succinct text for medical students both in temperate regions and in the tropics.

JOHN F. KESSEL

Manual of Clinical Mycology, by NORMAN F. CONANT, Ph.D., DAVID T. SMITH, M.D., ROGER D. BAKER, M.D., JASPER L. CALLAWAY, M.D., Duke University School of Medicine; and DONALD S. MARTIN, M.D., Communicable Disease Center, Georgia. 2d edition: 456 pp., with 202 figures, Philadelphia and London: W. B. Saunders Co., 1954. Price $6.50.

The second edition of this Manual is increased in size by more than 100 pages by the addition of 88 new illustrations, making a total of 202, and by three additonal chapters. The chapter on Actinomycosis has been separated into two chapters—one on Actinomycosis and the second on Nocardiosis. This perhaps is unwarranted. Two new chapters, one on tinea nigra palmaris and the other, a half page, is devoted to coniosporosis which is suggested as an allergic reaction rather than a true mycosis. The book is made up of 28 chapters including an appendix consisting of mycologic, pathologic, immunologic and dermatologic methods and a formulary. The increase in the number of pages, the additional information and the elaborate discussions of some of the mycoses appear to take this book out of the class of manuals. The lack of detailed information of some of the diseases, however, fails to place the book in the category of textbooks.

The authors particularly emphasize, perhaps unduly, immunity with detailed discussions of serology and hypersensitivity. There are several statements made in the book with which many might disagree. *Nocardia brasiliensis* is considered to be a partially acid-fast organism. Recent work indicates that this organism develops partial acid-fastness only when grown in milk medium which may cause non-acid-fast organisms to develop this property. The authors retain the name *Blastomyces* for the organism causing North American blastomycosis although admitting that the term is incorrect. To add confusion, the authors apply this generic terminology to the organisms causing South American blastomycosis to replace the term *Paracoccidioides* which is already well established and accepted. They further fail to distinguish the three species of this genus, *P. brasiliensis*, *P. cerebriformis* and *P. tenuis* but instead incorporate them in the one species, *Blastomyces brasiliensis*. On page 184, the statement is made "Since *C. albicans* is the only pathogenic member of the genus. . . ." This obviously is a misstatement since other species definitely can and do produce disease. The term maduromycosis is used to include infections caused by many higher fungi of different genera and species. This term should be limited to those lesions caused only by species of the genus *Madurella*. The term mycetoma should be applied as an all inclusive term for tumefactions of the skin with sinuses. The classification of the organisms causing chromomycosis (chromoblastomycosis) needs revision since the authors prefer to revert to the genus *Hormodendrum* formerly used for some of the species now thought to be better placed in the genus *Phialophora*. The section on the dermatomycoses is well illustrated and the descriptions of the lesions are good but a little brief. Dermatomycoses represent a great majority of fungus infections seen by the dermatologists who would benefit by greater detail particularly of those lesions caused by *Trichophyton rubrum*. On pages 330 and 332, microsporosis is listed as affecting only hair and skin. The nails may also be affected by species of *Microsporum*. There is a tendency to list several species as synonyms of one species. This has been done with species of the crateriform group, *Tricho-*

phyton tonsurans, *T. epilans*, *T. Sabouraudi* and *T. sulfureum*, all of which they consider as *T. tonsurans*. The important gross characteristics which are variable with these species are not considered worthy of specific rank. This the reviewer considers as unjustifiable oversimplification. In the table on page 330, *Trichophyton roseum* and *T. megnini* are listed as species of the rosaceum group. On page 343, *T. roseum* is considered as a synonym for *T. megnini* and the second species is *T. gallinae*. This should be corrected.

The Manual does not include all the known mycoses. Despite the various criticisms, however, the book is compact, well printed and illustrated and remarkably free of typographical errors. It contains a great deal of information useful to student and clinician alike.

MORRIS MOORE

Rh-Hr Blood Types. Applications in Clinical and Legal Medicine and Anthropology, by ALEXANDER S. WIENER, M.D., F.A.C.P., F.C.A.P., Senior Bacteriologist (Serology) to the Office of the Chief Medical Examiner of New York City. 763 pp., illustrated. New York, Grune & Stratton, 1954. Price $11.50.

This book, by a leading blood-group investigator and renowned serologist, has long been overdue. Clarification of the intricacies of the Rh-Hr blood types is badly needed. Disappointingly, however, this is merely a collection of 84 reprints of Dr. Wiener's numerous articles of the last dozen or more years, selected, not exclusively for scientific value but often to claim priority for ideas, to stress the merits of his special nomenclature, and to ridicule any other. Many of the brief case reports to which long discussions were appended could better have been combined in single more compact articles. The author's complete bibliography, listing 260 publications in the last twelve years, shows boundless energy but the articles themselves are often repetitious, apparently hastily written, and argumentative.

Specifically, of the 84 reprints reproduced here, about 10 presented important new data; another dozen might have been condensed into a few good articles; six were "letters to the editor" trying to refute someone else's viewpoint and present the author's; ten were simply directions for laboratory technics; and at least a dozen were almost solely contentious.

Dr. Wiener is at his best when he presents serologic experiments, and discusses forensic medicine and the genetics of the blood groups. In the clinical field, particularly regarding erythroblastosis fetalis and its treatment, he exhibits a surprising unscientific tendency to jump to conclusions and to draw inferences from small numbers of cases. When discussing nomenclature, he might well have been guided by Aesop's story (Fable #57) of the wind and the sun.

Had Dr. Wiener taken the time and trouble to prepare a condensed review of work on the Rh-Hr blood types, this might have been an important compendium. As it is, this is an unnecessarily lengthy book, if a set of bound reprints can be called a book. It cannot be recommended except possibly for historical value.

LOUIS K. DIAMOND

An Rh-Hr Syllabus. The Types and Their Applications. Modern Medical Monographs, 9, by ALEXANDER S. WIENER, M.D. F.A.C.P., F.C.A.P., Senior Bacteriologist (Serology) to the Office of the Chief Medical Examiner of New York City. 82 pp. illustrated. New York, Grune & Stratton, 1954, Price $3.75.

This book epitomizes the current concepts of the immunology of isohemagglutination. In abstract form it defines all of the terms and outlines the techniques involved in the manipulative procedure of the applied science of immunology referable to the field of the Rh-Hr phenomena. In no other single volume is a comparable amount of information available on the subject of applied immunology in the field of autohemagglutination. The table of contents and the index cover every known feature of the problem as of the date of publication. The text is clear and terse and the volume is *practical*.

The book, comprising 77 text pages, is divided into 8 chapters. The first of these deals with the fundamentals of immunology as applied to the field of isohemagglutination. Each subject introduced is carefully defined, and the techniques of testing are outlined. Appended is a table of the 8 Rh genotypes giving their frequency for the 7 major ethnic groups.

Chapter II deals with the specific properties and reactions of Rh antibodies. The various characteristics of the reactions and the techniques for demonstrating the agglutinating properties of the Rh antibodies are shown diagrammatically. In Chapter III the mechanism of the genetics and serologic reactions of the Rh-Hr genotypes and phenotypes is discussed. The theory of multiple allelomorphism and the calculation of Rh gene frequencies according to the author's nomenclature are given full description. In Chapter IV the basic features of hemolytic disease of the newborn are adequately reviewed. Unfortunately the exposition is at times unclear and the philosophy of hypothesis detracts from the observation of fact. The chapter on blood transfusion is the shortest the reviewer has had the privilege to see and by far the most informative on the subject. Wiener demonstrates his mastery in this field. In Chapter VI the phenomena of autosensitization are defined, and it is clear that the author bases his discussion on a large mass of observations.

In Chapter VII the anthropological features of gene inheritance and frequency is given, without the tempering influence of other authorities in the field. The final chapter on medico-legal applications of Rh-Hr phenomena recapitulates the basic features of isohemagglutination and indicates their application to an important field much too often confused.

The volume is a significant contribution to the subject of the Rh factor in isoimmunization. The author succeeds in expounding his concept which is rendered short of a classic only by his prejudices.

S. P. Lucia

Influenza: A Review of Current Research by Various Authors. World Health Organization Monograph Series, No. 20, 224 pages. Geneva: W.H.O., Palais des Nations, or Columbia University Press, International Documents Services, 2960 Broadway, New York 27, N. Y. Price: 17/6d, U.S. $2.50, Sw. fr. 10.—. French edition in preparation.

This monograph consists of nine papers prepared by distinguished investigators in the field of influenza. The ground covered is beyond that encompassed in the usual text on influenza, the intention of the monograph being to cover the more important aspects of current research and investigation and to present in detail information usually presented in a general fashion in textbooks or reviews.

Epidemiologists will find much interesting and well correlated information in the three chapters on epidemiology. Some thought-provoking information is provided by Hilleman *et al.* on the possibility of predicting the susceptibility of a population for a particular virus.

A description of the virus and its properties and characteristics is presented by von Magnus, and recent work on the somatic and genetic aspects of influenza virus is outlined by Burnet. Burnet's chapter has high scientific interest because of the basic information recently obtained in the laboratory and the likelihood that similar techniques may provide data of fundamental importance in other viruses. The laboratory methods used in the study of influenza virus and for diagnostic and epidemiologic studies are very concisely and lucidly outlined by Lepine; this presentation will be found very useful to the laboratory worker since it presents in one chapter a summary of all the current procedures utilized in this field.

The important subject of prophylaxis against influenza is ably covered by Francis, who summarizes and evaluates the earlier efforts on vaccination and attempts to develop a vaccine. Recent work on influenza vaccines is also clearly presented and analyzed, and it is concluded that there is every reason to believe that effective prophylaxis against the range of influenza viruses is attainable.

Not the least important part of the monograph is the inclusion of a select bibliography on

influenza, a bibliography which is especially appreciated because it provides the interested reader with access to highly pertinent publications on various aspects of the influenza problem.

EDWIN H. LENNETTE

W.H.O. Expert Committee on Poliomyelitis, First Report. Technical Report Series No. 81. 69 pp., W.H.O. Geneva, 1954. Distributed by Columbia University Press, International Documents Service, 2960 Broadway, New York 27, New York. Price $.50, 3/6, Sw. fr. 2.

This Expert Committee Report provides much valuable information on a disease which is a health problem of increasing importance and general interest. Since it is a First Report it will be of service to many readers, since it contains a review of recent advances that have been made as well as an account of present concepts of the essential nature of this disease. The subjects covered include the epidemiology of poliomyelitis, its clinical features, portals of entry and distribution of the virus in the body, immunization of the disease, advances in laboratory techniques, and control measures.

The present concept of transmission in epidemic areas is one of spread during close personal association with infected persons who are excreting virus in the feces and pharyngeal secretions. It is now thought that the portal of entry is usually the mouth, and that the primary site of infection is in the pharynx and the rest of the alimentary tract. Hope for the control of the disease through immunizing measures now runs high. The Report, which is dated April, 1954, contains a section on gamma globulin and also deals in a preliminary way with the early vaccination procedure, which it rightly considers still in the experimental stage.

L. W. HACKETT

ERRATUM

In the paper on Arsthinol, page 262, this journal Volume 3, Number 2, March 1954, the author's name Edward B. Rossman should read Edward B. Grossman.

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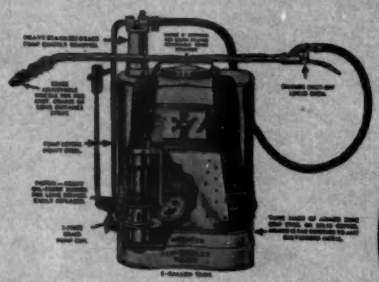

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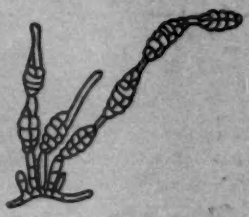
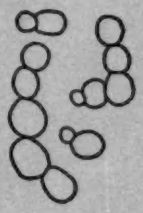


DIAGNOSTIC

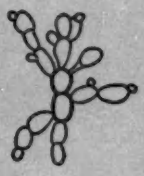
. . . for the isolation, identification and cultivation of pathogenic fungi. These media are also widely used in phytopathological studies. Several are neutral in reaction, giving optimum conditions for growth of a variety of fungi. The following may be prepared as selective media by the adjustment of reaction, addition of antibiotics or other agents:



Bacto-Brain Heart Infusion Agar
Bacto-Sabouraud Dextrose Agar
Bacto-Sabouraud Maltose Agar
Bacto-Littman Oxgall Agar
Bacto-Mycological Agar
Bacto-Mycological Broth
Bacto-Corn Meal Agar
Bacto-Corn Meal Agar with Dextrose
Bacto-Bean Pod Agar
Bacto-Prune Agar
Bacto-Lima Bean Agar

CONTROL

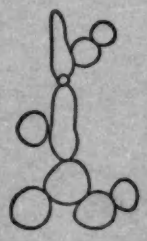
. . . for sanitary and sterility procedures as well as for general use in mycological procedures:



Bacto-Sabouraud Maltose Broth
Bacto-Sabouraud Liquid Medium
Bacto-Malt Extract
Bacto-Malt Agar
Bacto-Neurospora Culture Agar
Bacto-Potato Dextrose Agar
Bacto-Mildew Test Medium
Bacto-W.L. Nutrient Medium
Bacto-W.L. Differential Medium

CLASSIFICATION

. . . and nutritional studies of fungi:



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Bacto-Yeast Nitrogen Base
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